

03/30/99
JC520 U.S. PTO

Patent
241/08
JC542 U.S. PTO
09/28/99
03/30/99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Anticipated Classification of this application:

Class _____ Subclass _____

Prior application: _____

Examiner: Tung

Art Unit: 1644



22249

PATENT TRADEMARK OFFICE

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D. C. 20231

FILING UNDER 37 CFR § 1.53(B)

This is a request for filing for a

☐ continuation ☐ divisional ☒ continuation-in-part (CIP)

application under 37 CFR § 1.53(b) of pending prior application Serial No. 09/058,331 filed on April 9, 1998 of

Robert Lawton, Brion Mermer, Greg Francoeur

for: **SPECIFIC BINDING PROTEIN FOR TREATING CANINE ALLERGY**

I. PAPERS ENCLOSED HEREWITH FOR FILING:

37 Page(s) of Specification

14 Page(s) Claims

1 Page(s) Abstract

15 Sheets of Drawings ☒ Informal ☐ Formal

 Page(s) of Declaration and Power of Attorney

 Small Entity Statement

LA-81784.1

CERTIFICATE OF MAILING
(37 C.F.R. §1.10)

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as 'Express Mail Post Office To Addressee' in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

EM350826021US

Express Mail Label No.

March 30, 1999

Date of Deposit

RUDY REYES

Name of Person Mailing Paper

[Signature]
Signature of Person Mailing Paper

- ☐ Pursuant to 37 CFR 1.63(d)(1), a newly executed oath or declaration is not required.
- ☐ A newly executed oath or declaration is filed herewith
- ☐ I hereby state that the amendment referred to in the declaration filed to complete the prior application, in accordance with the requirements of 37 CFR § 1.53(b), did not introduce new matter therein.

II. AMENDMENTS

- ☐ Cancel in this application original Claims _____ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
- ☐ A Preliminary Amendment is enclosed. (Claims added by Amendment must be numbered consecutively beginning with the number next following the highest numbered original claim in the prior application.)

III. INFORMATION DISCLOSURE STATEMENT

- ☐ An Information Disclosure Statement, PTO 1449, and references are submitted herewith.

IV. PETITION FOR SUSPENSION OF PROSECUTION FOR THE TIME TO FILE AN AMENDMENT

- ☐ There is provided herewith a PETITION FOR SUSPENSION OF PROSECUTION FOR THE TIME NECESSARY TO FILE AN AMENDMENT (NEW APPLICATION FILED CONCURRENTLY).

V. FEE CALCULATION

BASIC FILING FEE:					\$760.00
Total Claims	-	20	=	0	x \$18.00 \$0.00
Independent Claims	-	3	=	0	x \$78.00 \$0.00
Multiple Dependent Claims	\$260	(if applicable)		<input type="checkbox"/>	\$0.00
Surcharge 37 CFR § 1.16(e)	\$130	(if applicable)		<input type="checkbox"/>	\$0.00
TOTAL OF ABOVE CALCULATIONS					\$760.00
Reduction by ½ for Filing by Small Entity. Note 37 CFR §§ 1.9, 1.27, 1.28. If applicable, Verified Statement must be attached.					<input type="checkbox"/> \$0.00
Misc. Filing Fees (Recordation of Assignment)					\$0.00
TOTAL FEES					\$760.00

- ☒ The fee for extra claims is not being paid at this time.

VI. SMALL ENTITY STATUS

A Verified Statement to establish small entity under 37 CFR §§ 1.9 and 1.27:

- ☐ is attached
- ☐ has been filed in the prior application and such status is still proper and desired. [37 CFR § 1.28(a)]

Filing Fee Calculation (50% of above) _____

VII. DRAWINGS

[NOTE: DO NOT CHECK THIS IF PRIOR CASE IS NOT TO BE ABANDONED.]

- ☐ Transfer the drawings from the prior application to this application and, subject to Item 16 below, abandon said prior application as of the filing date accorded to this application. A duplicate copy of this request is enclosed for filing in the prior application file.
[May only be used if signed by (1) applicant, (2) assignee of record or (3) attorney or agent of record and before payment of issue fee. 37 CFR § 1.138.]
- ☐ Transfer the following sheet(s) of drawings from the prior application to this application.
- ☐ New drawings are enclosed ☐ formal ☐ informal

VIII. PRIORITY - 35 USC § 119

- ☐ Priority of application Serial No. _____ filed on _____ in Country is claimed under 35 USC § 119.
- ☐ The certified copy has been filed in prior U.S. application Serial No. _____ on _____.
- ☐ The certified copy will follow.

IX. RELATE BACK - 35 USC § 120

- ☒ Amend the Specification by inserting before the first line the sentence:
This is a continuation of co-pending application Serial No. 09/058,331 filed April 9, 1998.

X. INVENTORSHIP STATEMENT

- ☒ With respect to the prior co-pending U.S. application from which this application claims benefit under 35 USC § 120, the inventor(s) in this application is (are):

- ☒ the same
- ☐ less than those named in the prior application and it is requested that the following inventor(s) identified above for the prior application be deleted:
- ☒ The inventorship for all the claims in this application are:
- ☒ the same
- ☐ not the same, and an explanation, including the ownership of the various claims at the time the last claimed invention was made, is submitted.

XI. ASSIGNMENT

- ☒ The prior application is assigned of record to IDEXX LABORATORIES, INC..
- ☐ An Assignment of the invention to _____ is attached.

XII. FEE PAYMENT BEING MADE AT THIS TIME

- ☐ Not attached. No filing fee is submitted. [This and the surcharge required by 37 CFR § 1.16(e) can be paid subsequently.]
- ☐ Attached.
- ☐ Filing fees. -----
- ☐ Recording assignment. [\$40.00 37 CFR § 1.21(h)(1)] -----
Petition fee for filing by other than all the inventors or
person on behalf of the inventor where inventor
refused to sign or cannot be reached.
[\$130.00; 37 CFR §§ 1.47 and 1.17(h)] -----
- ☐ Petition fee to Suspend Prosecution for the Time -----
Necessary to File an Amendment (New Application
Filed Concurrently.)
[\$130.00; 37 CFR §§ 1.103 and 1.17(i)] -----
- ☐ For processing an application with a specification in a -----
non-English language.
[\$130.00; 37 CFR §§ 1.52(d) and 1.17(k)] -----
- ☐ Processing and retention fee. -----
[\$130.00; 37 CFR §§ 1.53(f) and 1.21(l)] -----

Total Fees Enclosed -----

XIII. METHOD OF PAYMENT OF FEES

- ☐ Attached is a check in the amount of ____.
- ☐ Charge Deposit Account No. **12-2475** in the amount of ____.

XIV. AUTHORIZATION TO CHARGE ADDITIONAL FEES

The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Deposit Account No. **12-2475**:

- ☐ 37 CFR § 1.16(a) (filing fees)
- ☐ 37 CFR § 1.16(b) (presentation of extra claims)
- ☐ 37 CFR § 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)
- ☐ 37 CFR § 1.17 (application processing fees)
- ☐ 37 CFR § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR § 1.311(b))

XV. INSTRUCTIONS AS TO OVERPAYMENT

- ☐ Credit Deposit Account No. **12-2475**.
- ☐ Refund

XVI. POWER OF ATTORNEY

- ☐ The power of attorney in the prior application is to ____.
- ☒ The power of attorney in the prior application is to the registered attorneys listed below and members of or associates in the law firm of **LYON & LYON LLP**, 633 West Fifth Street, 47th Floor, Los Angeles, California 90071, Registration No. 11,611, whose members are registered to practice in the U.S. Patent and Trademark office:

Roland N. Smoot, Reg. No. 18,718
Conrad R. Solum, Jr., Reg. No. 20,467
James W. Geriak, Reg. No. 20,233
Robert M. Taylor, Jr., Reg. No. 19,848
Samuel B. Stone, Reg. No. 19,297
Douglas E. Olson, Reg. No. 22,798
Robert E. Lyon, Reg. No. 24,171
Robert C. Weiss, Reg. No. 24,939
Richard E. Lyon, Jr., Reg. No. 26,300
John D. McConaghy, Reg. No. 26,733
William C. Steffin, Reg. No. 26,811
Coe A. Bloomberg, Reg. No. 26,605
J. Donald McCarthy, Reg. No. 25,119
John M. Benassi, Reg. No. 27,483
James J. Shalek, Reg. No. 29,749
Allan W. Jansen, Reg. No. 29,035
Robert W. Dickerson, Reg. No. 29,914
Roy L. Anderson, Reg. No. 30,240
David B. Murphy, Reg. No. 31,125

James C. Brooks, Reg. No. 29,898
Jeffrey M. Olson, Reg. No. 30,790
Steven D. Hemminger, Reg. No. 30,755
Jerrold B. Reilly, Reg. No. 32,293
Paul H. Meier, Reg. No. 32,274
John A. Rafter, Jr., Reg. No. 31,653
Kenneth H. Ohriner, Reg. No. 31, 646
Mary S. Consalvi, Reg. No. 32,212
Lois M. Kwasigroch, Reg. No. 35,579
Lawrence R. LaPorte, Reg. No. 38,948
Robert C. Laurensen, Reg. No. 34,206
Carol A. Schneider, Reg. No. 34,923
Hope E. Melville, Reg. No. 34,874
Michael J. Wise, Reg. No. 34,047
Richard J. Warburg, Reg. No. 32,327
Kurt T. Mulville, Reg. No. 37,194
Theodore S. Maceiko, Reg. No. 35,593
Bruce G. Chapman, Reg. No. 33,846
F. T. Alexandra Mahaney, Reg. No. 37,668
Reg. No. _____

- ☒ The power appears in the original papers in the prior application.
- ☐ The power does not appear in the original papers, but was filed on _____ in this application.
- ☐ A new power has been executed and is attached.
- ☒ Address all future communications to:

LYON & LYON LLP
633 West Fifth Street, 47th Floor
Los Angeles, California 90071
(213) 489-1600
Attention: **Carol A. Schneider, Ph.D., J.D.**

XVII. MAINTENANCE OF CO-PENDENCY OF PRIOR APPLICATION

- ☐ A petition, fee and response has been filed to extend the term in the pending **prior** application until _____. A copy of the petition for extension of time in the **prior** application is attached.

XVIII. CONDITIONAL PETITIONS FOR EXTENSION OF TIME IN PRIOR APPLICATION

- ☐ A conditional petition for extension of time is being filed in the pending **prior** application. A copy of the conditional petition for extension of time in the **prior** application is attached.

XIX. ABANDONMENT OF PRIOR APPLICATION

- ☒ Please abandon the prior application at a time while the prior application is pending or when the petition for extension of time or to revive in that application is granted and when this application is granted a filing date so as to make this application co-pending with said prior application. At the same time, please add the words "now abandoned" to the amendment of the specification set forth in Item 2 above.

Respectfully submitted,

LYON & LYON LLP

Dated: March 30, 1999

By:

Carol A. Schneider
Carol A. Schneider, Ph.D., J.D.
Reg. No. 34,923

633 West Fifth Street, Suite 4700
Los Angeles, California 90071-2066
(213) 489-1600

Enclosures

Name of Assignee

Address of Assignee

Title of person authorized to sign on behalf of assignee

Assignment recorded in PTO on _____, Reel _____, Frame _____

241/088
PATENTSPECIFIC BINDING PROTEINS FOR TREATING CANINE ALLERGY

This application is a continuation-in-part of
copending application United States Serial Number 09/058,331,
5 filed April 9, 1998, which is incorporated herein in its
entirety.

FIELD OF THE INVENTION

This invention concerns peptides. More particularly, the
10 invention concerns compositions for administration to dogs,
which actively provide immunity to the dog's immunoglobulin E
molecules.

BACKGROUND ART

15 It is estimated that up to 30% of all dogs suffer from
allergies or allergy-related skin disorders. Specifically,
allergic dermatitis has been estimated to affect between 3 and
15% of the entire canine population. Given the prevalence of
allergies in dogs, there is a need to develop methods and
20 compositions to properly diagnose and treat canine allergies.

The substances most likely to cause an allergic reaction
vary from species to species. Common canine allergens include
fleas, pollens, molds and dust. Allergy to fleas is believed
to be the most common dog allergy. Typically, a flea's saliva
25 is the allergen, and a single fleabite can cause substantial
itching. An additional form of allergy in dogs is termed
atopy. Atopy is a condition where a dog is allergic to

inhalants such as pollens, molds or microscopic mites such as are found in house dust.

Antibody molecules play a role in allergic manifestations. In mammals, antibody molecules are classified into various isotypes referred to as IgA, IgD, IgE, IgG, and IgM. Antibody molecules consist of both heavy and light chain components. The heavy chains of molecules of a given isotype have extensive regions of amino acid sequence homology, and conversely have regions of difference from antibodies belonging to other isotypes. The shared regions of the heavy chains provide members of each isotype with common abilities to bind to certain cell surface receptors or to other macromolecules, such as complement. These heavy chain regions, therefore, serve to activate particular immune effector functions. Accordingly, separation of antibody molecules into isotypes serves to separate the antibodies according to a set of effector functions that they commonly activate.

In humans and dogs, immunoglobulin E (hereinafter IgE) is involved in allergy. Thus, IgE is the antibody type that is understood to be an important mediator of allergic responses, including Type I immediate hypersensitivity.

IgE molecules bind to mast cells and basophils. This binding occurs when the Fc region of the IgE molecule is bound to Fc receptors on the mast cells. When such bound IgE antibodies then bind to an allergen, the allergen cross-links multiple IgE antibodies on the cell surface. This cross-linking mediates Type I immediate hypersensitivity reactions and causes release of histamines and other molecules that produce symptoms associated with allergy.

Monoclonal antibodies having different degrees of sensitivity to canine IgE and IgG have been identified. (DeBoer, et al. Immunology and Immunopathology 37, 183-199 (1993).) DeBoer, et al. identified several monoclonal antibodies which had cross reactivity between IgG and IgE. (See, e.g., DeBoer, et al., Table 4 and accompanying text.) Three monoclonal antibodies (A5, D9, and B3) were identified by DeBoer et al., as having some affinity for canine IgE. Of the monoclonal antibodies identified in DeBoer et al., antibody D9 appeared to have the greatest degree of neutralization of Prausnitz-Kustner reactivity for atopic dog serum. In the context of canine allergy, DeBoer et al. proposed use of their monoclonal antibodies (MAbs) in the use of antigen-specific IgE ELISA, and for quantifying canine IgE. Additionally, they proposed use of their MAbs for immunostaining of Western Blot assays, to evaluate the molecular specificity of IgE antibodies, as well as for *in vitro* studies on degranulation of mast cells.

In humans the serum level of total IgE is diagnostic of allergic disease. To explore the possibility that the serum level of IgE might also be diagnostic of allergy in dogs, several studies were performed. (Hill and DeBoer Am. J. Vet. Res., (July 1994) 55(7), 944-48). Publications following the DeBoer article used a monoclonal antibody designated D9 in an ELISA assay having the following configuration: D9 was bound to a substrate, antibodies were captured by D9 and then D9 having a marker was used to flag the captured antibody. The Hill and DeBoer ELISA was used to establish the total amount of IgE in canine serum in an effort to diagnose canine allergy. In contrast to humans, the quantity of IgE determined

to exist in canine circulation was of no use whatsoever in the diagnosis of allergy in dogs. (See, e.g., Abstract and Discussion Sections of Hill and DeBoer) This finding was in direct contrast to the situation in human immunology.

5 This divergent diagnostic result based on levels of IgE in humans compared to such levels in dogs, points out the difficulty of any attempt to correlate data between animals of two different genera. This difficulty is further exacerbated by the fact that dogs can be allergic to a different set of
10 antigens than humans are. Fleas, for instance, are a severe problem for dogs, but not humans. Furthermore, in instances where dogs and humans appear to be allergic to the same allergen extract, studies by doctors Esch and Greer of Greer Laboratories, have indicated that the specific allergens in an
15 allergen extract which produce canine disease are not necessarily the same allergens that produce disease in humans. For example, it is known that the immunodominant components of dust mite extracts are different in dogs than in humans.

 The genomic sequences encoding human and murine IgE heavy
20 chain constant region are known (For example, see Ishida et al., "The Nucleotide Sequence of the Mouse Immunoglobulin E Gene: Comparison with the Human Epsilon Gene Sequence", EMBO Journal 1,1117-1123 (1982). A comparison of the human and murine genes shows that they possess 60% homology within
25 exons, and 45-50% homology within introns, with various insertions and deletions.

 Patel et al. published the nucleotide and predicted amino acid sequence for exons 1-4 of the heavy chain constant region of canine IgE in the article entitled "Sequence of the Dog
30 Immunoglobulin Alpha and Epsilon Constant Region Genes,"

Immunogenetics 41, 282-286 (March 22, 1995). The complete sequence of the canine IgE heavy chain constant region, with membrane bound portions encoded by exons 5 and 6 are disclosed in copending applications Serial Numbers 08/800,698 filed
5 February 14, 1997, 09/146,400 filed September 3, 1998, and 09/146,617 filed September 3, 1998.

Because IgE is believed to mediate allergic symptoms, it may be desirable to decrease IgE levels as a mechanism for alleviating allergic symptoms. However, a patient's own IgE
10 molecules are self-proteins, and immune responses to such proteins are usually suppressed. The suppression of immune responses to self-proteins, i.e., tolerance to self-antigens, is hypothesized to occur in a number of ways.

The current hypothesis for suppression of T cells
15 directed to self-antigens, involves an induction of "clonal deletion" of such T cells in the thymus, whereby T cell receptors which might recognize self-peptides in association with MHC molecules are eliminated, and only those which recognize foreign peptide and MHC molecules are allowed to
20 expand. In addition, suppressor T cells may also exist which prevent the induction of immune responses to self-proteins.

In contrast to the situation with T cells, it is believed that there are many B cells which express receptors (i.e., surface immunoglobulin) for self-proteins, and that the reason
25 these cells do not produce antibodies to self-proteins is because the T cells required for the antigen presentation to the B cell are normally missing.

A B cell which recognizes epitopes (antigen-binding sites) on a patient's own IgE antibodies is capable of
30 generating antibodies, generally IgG, directed to this self-

antigen, i.e., IgE. The existence of such B cells, therefore, presents a unique opportunity to induce the production of auto-antibody responses. There is an unmet need for such antibodies in order to treat allergic disease.

5 The hypothesis regarding "antigen presentation" involves: the recognition of antigen by surface immunoglobulin on the B cell, the internalization and processing of this antigen, the association of peptides derived from the antigen with MHC molecules expressed on the surface of the B cell, and then,
10 the recognition of the associated antigen peptide and MHC molecules by a particular T cell. The T-cell:B-cell interaction then leads to signal transduction in both cells and the synthesis and elaboration of soluble cytokines which eventually result in antibody production by the B cell.

15 Thus, in most circumstances, only when an antigen is foreign does an immune response occur; otherwise the internalization and processing of self-proteins would regularly lead to the presentation of self peptide-MHC complexes to T cells and thereby lead to autoimmune
20 antibodies.

 Therefore, in order to induce an antibody response to a self-peptide, such as IgE, the immune system must be manipulated so as to allow an auto-reactive B cell to become an antibody-secreting B cell. There is an unmet need to
25 manipulate the immune system in this way, particularly in the context of allergic disease.

 In general, there are several known approaches for generating antibodies to peptide antigens. For example, multiple antigenic peptides (MAPs), introduced by Dr. James
30 Tam (Tam, J.P., (1988) Proc. Natl. Acad. Sci. U.S.A. 85,
LA-81681.1

5409-5413), have demonstrated several advantages for inducing anti-peptide antibodies. The MAP approach is an improved alternative to the conventional technique of conjugating a peptide antigen to a protein carrier. One of the primary
5 limitations associated with the use of protein carriers is the large mass of the carrier relative to the attached peptide antigen. This relative size disparity may result in a low ratio of anti-peptide antibodies compared to anti-carrier antibodies. MAPs typically have 4 or 8 peptide arms branching
10 out from a lysine core matrix as depicted in Fig.1 A-B. The peptide antigen is conjugated to each arm. Thus there is a much higher ratio of antigen to carrier molecule in a MAP system compared to traditional protein conjugation. This design maximizes the concentration of the antigen for a
15 specific immunogenic response. Moreover, the central lysine core of the MAP-peptide has been shown to be non-immunogenic. (Tam, J.P., Proc. Natl. Acad. Sci. U.S.A. 85, 5409-5413 (1988); Posnett, D.N., McGrath, H., and Tam, J.P., J. Biol. Chem. 263, 1719-1725 (1988)) Therefore, antibodies induced to
20 MAP-peptides are a direct response to the antigen. Accurate knowledge of the chemical composition, structure, and quantity of the peptide prior to immunization is possible by directly synthesizing the antigen onto the branching lysine core. Also, because the MAP approach removes the need to conjugate
25 peptides to carrier proteins, which may alter the antigenic determinants, chemical ambiguity is eliminated. Thus MAPs are believed to induce antibody responses of high purity, increased avidity, accurate chemical definition, and improved safety.

Fmoc MAP resins (available from Applied Biosystems, Foster City, Calif.) are Fmoc-compatible resins connected to a small core matrix of branching lysine residues. The core matrix comprises several levels of lysine residues attached to the previous lysine at both the N- α and N- ϵ amino groups, as depicted in Figure 1A-B.

MAP-peptides used in experimental vaccine design have elicited high titers of anti-peptide antibodies that recognize the native protein. (Tam, J.P., (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 5409-5413; Posnett, D.N., McGrath, H., and Tam, J.P., (1988) J. Biol. Chem. 263, 1719-1725; Auriault, C., Wolowczuk, I., Gras-Masse, H., Maguerite, M., Boulanger, D., Capron, A., and Tartar, A., (1991) Peptide Res.4, 6-11). Additionally, increased sensitivity and reliability of antibody-antigen interactions in solid-phase immunoassays have been observed with MAP-peptides due to enhanced coating capacity and avidity. (Tam, J.P., and Zavala, F., (1989) J. Immunol. Meth., 124, 53-61).

An additional approach that is known to be useful for generating antibodies to peptide antigens involves placing multiple copies of peptides on the surface of plant virus particles. EPICOAT™ technology (Axis Genetics plc, Cambridge, England) is one such example. The EPICOAT™ technology is based on chimeric virus particle (CVP) technology that utilizes the recombinant genetic modification of plant viruses.

The EPICOAT™ technology involves insertion of a small portion of a foreign protein (a peptide) into a plant virus in such a way that multiple copies of the peptide are displayed on the surface of the virus particle. The EPICOAT™ technology

is currently based on the cow pea mosaic virus (CPMV) a plant virus that infects the cow pea plant, also known as the "black-eyed" bean. The unmodified CPMV particle is icosahedral, and about twenty-eight nanometers (nm.) in diameter. CPMV particles are composed of two proteins, referred to as the large and small coat proteins. Studies have revealed a site within the small coat protein which allows presentation of a foreign peptide in a prominent position on the virus surface, whereby up to sixty copies of a particular peptide can be presented on each virus particle.

With the EPICOAT™ technology, DNA copies of the plant virus's genetic material are used. A minute quantity of the DNA encoding the virus protein, including the inserted foreign peptide, is applied to the leaves of young cow pea plant, along with an abrasive powder. Upon gentle rubbing, DNA enters the leaves and utilizes the plant's own cellular mechanisms to initiate generation of functional virus particles. The virus replicates within the inoculated leaves and spreads throughout the growing plant.

After two to three weeks, leaf material containing large quantities of the virus is harvested. Chimeric virus particles (CVPs) are isolated by centrifugation and selective precipitation of homogenized plant material. Between 1 to 2 grams of CVPs can be obtained per kilogram fresh weight of leaf material. Cow pea plants are readily grown in abundance in controlled environments, allowing generation of large quantities of CVPs.

It has been reported that peptides of up to thirty-six amino acids in size have been successfully incorporated into

CVPs. The resulting particles are extremely robust with a thermal inactivation point of 65°C. The CVPs have been shown to withstand acidic pH as well as protein degrading enzymes. It has been reported that the expressed peptides are capable of eliciting specific immune responses in animals. It has been hypothesized that surface presentation of peptides may enhance the recognition by the host immune system, and may provide a route for development of recombinant sub-unit vaccines and immuno-therapeutics.

10 DISCLOSURE OF THE INVENTION

Disclosed is a specific binding protein which specifically binds to native canine free or B cell-bound IgE exon 3, and which does not bind to IgE exon 3 when the IgE is bound to receptor on a mast cell. The surface-bound IgE can be IgE expressed on the surface of a canine B cell.

Disclosed is a specific binding protein which specifically binds to an isolated and purified peptide comprising a leucine positioned two peptide bonds away from a tyrosine-arginine pair; e.g., SEQ ID NO:1 leucine-blank-blank-tyrosine-arginine, SEQ ID NO:2 tyrosine-arginine-blank-blank-leucine, or SEQ ID NO:3 leucine-blank-blank-tyrosine-arginine-blank-blank-leucine. The peptide can consist of from 5 to 71 amino acids.

Disclosed is an antibody which binds to a defined epitope and which is raised to an isolated and purified peptide comprising an amino acid sequence, or a conservative variant thereof, which comprises: SEQ ID NO:4 Thr-Leu-Leu-Glu-Tyr-Arg-Met; also disclosed is a recombinant binding molecule which

specifically binds to the defined epitope bound by the antibody.

Disclosed is an antibody which binds to a defined epitope and which is raised to an isolated and purified peptide comprising an amino acid sequence, or a conservative variant thereof, which comprises: SEQ ID NO:5 Gly-Met-Asn-Leu-Thr-Trp-Tyr-Arg-Glu-Ser-Lys; also disclosed is a recombinant binding molecule which specifically binds to the defined epitope bound by the antibody.

Also disclosed is a specific binding protein which is raised to a multiply antigenic peptide comprising multiple copies of an isolated and purified peptide which comprises a leucine positioned two peptide bonds away from a tyrosine-arginine pair; the specific binding protein specifically binds to a defined epitope. The isolated and purified peptide can be from 5-71 amino acids. Also disclosed is a recombinant binding molecule which specifically binds to the defined epitope bound by the binding protein.

Disclosed is a specific binding protein which specifically binds to an isolated and purified peptide comprising a cysteine positioned two peptide bonds away from a proline-histidine pair positioned three peptide bonds from a cysteine. The peptide can have the form: SEQ ID NO:6 cysteine-blank-blank-proline-histidine-blank-blank-blank-cysteine. The cysteines may form a covalent bond through a reduction reaction, cyclizing the peptide and becoming cystine. The peptide can comprise from 9 to 71 amino acids.

Disclosed is an antibody that binds to a defined epitope and which is raised to an isolated and purified peptide that has the amino acid sequence, or a conservative variant

thereof, which comprises: SEQ ID NO:7 serine-valine-threonine-leucine-cysteine-proline-asparagine-proline-histidine-isoleucine-proline-methionine-cysteine-glycine-glycine-glycine. The cysteines may form a covalent bond through a reduction reaction, cyclizing the peptide and becoming cystine. Also disclosed is a recombinant binding molecule that specifically binds to the defined epitope bound by the antibody.

Disclosed is an antibody that binds to a defined epitope and which is raised to an isolated and purified peptide that has the amino acid sequence, or a conservative variant thereof, which comprises: SEQ ID NO:8 serine-alanine-cysteine-proline-asparagine-proline-histidine-asparagine-proline-tyrosine-cysteine-glycine-glycine-glycine. The cysteines may form a covalent bond through a reduction reaction, cyclizing the peptide changing the amino acid to cystine. Also disclosed is a recombinant binding molecule that specifically binds to the defined epitope bound by the antibody.

Disclosed is a specific binding protein that specifically binds to an isolated and purified peptide comprising a cysteine positioned one peptide bond from a proline-histidine pair, positioned one peptide bond from a proline, positioned two peptide bonds from a cysteine; the peptide can have the form: SEQ ID NO:9 cysteine-blank-proline-histidine-blank-proline-blank-blank-cysteine. The cysteines may form a covalent bond through a reduction reaction, cyclizing the peptide and becoming cystine. The peptide can comprise from 9 to 71 amino acids.

Disclosed is an antibody that binds to a defined epitope and which is raised to an isolated and purified peptide that

has the amino acid sequence, or a conservative variant thereof, which comprises: SEQ ID NO:10 serine-alanine-cysteine-histidine-proline-histidine-leucine-proline-lysine-serine-cysteine-glycine-glycine-glycine. The cysteines may
5 form a covalent bond through a reduction reaction, cyclizing the peptide and becoming cystine. Also disclosed is a recombinant binding molecule that specifically binds to the defined epitope bound by the antibody.

Specific binding proteins in accordance with the
10 invention can be antibodies, either polyclonal or monoclonal. For example the monoclonal antibody can be 8H.8 or 15A.2. The antibody in accordance with the invention will bind a defined epitope; also disclosed are recombinant binding molecules which specifically binds to the defined epitope bound by an
15 antibody in accordance with the invention.

Also disclosed is a method for treatment or prophylaxis for canine allergy, said method comprising: providing a specific binding molecule in accordance with the invention; and, administering the provided binding molecule to a dog.
20 The method can comprise a step of mixing the provided binding molecule with a diluent prior to the administering step, and wherein the administering step comprises administering the mixture of the molecule and the diluent. A step of administering a booster dose of the binding molecule can
25 follow the administering step.

DEFINITIONS

Amino Acids:

AMINO ACID ABBREVIATIONS		
Amino Acid	One-Letter Symbol	Three-Letter Symol
alanine	A	ala
arginine	R	arg
asparagine	N	asn
aspartic acid	D	asp
cysteine	C	cys
glutamic acid	E	glu
glutamine	Q	gln
glycine	G	gly
histidine	H	his
isoleucine	I	ile
leucine	L	leu
lysine	K	lys
methionine	M	met
phenylalanine	F	phe
proline	P	pro
serine	S	ser
threonine	T	thr
tryptophan	W	trp
tyrosine	Y	tyr
valine	V	val

5 *cDNA clone:* A duplex DNA sequence representing an RNA, carried in a cloning vector.

Cloning: The selection and propagation of a single DNA species.

Cloning Vector: A plasmid, phage DNA or other DNA
10 sequence, able to replicate in a host cell and capable of carrying exogenously added DNA sequence for purposes of amplification or expression of the added DNA sequence.

Codon: A triplet of nucleotides that represents an amino acid or termination signal.

15 *Conservative variants:* Conservative variants of nucleotide sequences include nucleotide substitutions that do

not result in changes in the amino acid sequence encoded by such nucleotides, as well as nucleotide substitutions that result in conservative amino acid substitutions, e.g., amino acid substitutions which do not substantially affect the character of the polypeptide translated from said nucleotides. For example, the character of a peptide derived from IgE is not substantially affected if the substitutions do not preclude specific binding of the peptide to canine IgE receptor or other canine IgE binding ligands.

Conservative variants of amino acid sequences include amino acid substitutions or deletions that do not substantially affect the character of the variant polypeptide relative to the starting peptide. For example, polypeptide character is not substantially affected if the substitutions or deletions do not preclude specific binding of the variant peptide to a specific binding partner of the starting peptide. The term mimotope refers to a conservative variant of an amino acid sequence, to which antibody specificity has been raised. The mimotope comprises a variant of the epitope of the starting peptide such that it is able to bind antibodies that cross-react with the original epitope.

DNA Sequence: A linear series of nucleotides connected one to the other by phosphodiester bonds between the 3' and 5' carbons of adjacent pentoses.

Expression: The process undergone by a structural gene to produce a polypeptide. It is a combination of transcription and translation.

Expression Control Sequence: A DNA sequence of nucleotides that controls and regulates expression of structural genes when operatively linked to those genes.

Exon: A contiguous region of DNA encoding a portion of a polypeptide. Reference to any exon, e.g. "DNA sequence of exon 6", refers to the complete exon or any portion thereof.

Genome: The entire DNA of a substance. It includes
5 *inter alia* the structural genes encoding for the polypeptides of the substance, as well as operator, promoter and ribosome binding and interaction sequences such as the Shine-Dalgarno sequences.

Nucleotide: A monomeric unit of DNA or RNA consisting of
10 a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The four DNA bases are adenine ("A"), guanine ("G"), cytosine ("C") and thymine ("T"). The four RNA bases are A, G, C and uracil ("U"). A and G are purines, and C, T, and U are pyrimidines.

15 *Phage or Bacteriophage:* Bacterial virus, many of which include DNA sequences encapsidated in a protein envelope or coat ("capsid").

Plasmid: An autonomous self-replicating extrachromosomal circular DNA.

20 *Polymerase Chain Reaction (PCR):* A method of amplifying a target DNA sequence contained in a mixture of DNA sequences, by using oligonucleotide primers that flank the target DNA sequence for repeated cycles of DNA synthesis of the target DNA sequence.

25 *Polypeptide:* A linear series of amino acids connected one to the other by peptide bonds between the α -amino and carboxyl groups of adjacent amino acids.

Reading Frame: The grouping of codons during translation of mRNA into amino acid sequences. For example, the sequence

GCTGGTGTAAG may be translated in three reading frames or phases, each of which affords a different amino acid sequence:

GCT GGT TGT AAG-Ala-Gly-Cys-Lys

G CTG GTT GTA AG-Leu-Val-Val

5 GC TGG TTG TAA A-Trp-Leu-(STOP).

Recombinant DNA Molecule: A hybrid DNA sequence comprising at least two nucleotide sequences, the first sequence not normally being found together in nature with the second.

10 *Specific binding:* Binding of one substance to another at greater binding affinity than background binding. Two substances that exhibit specific binding are referred to as specific binding partners, or as a specific binding pair. An antibody and its antigen are one example of a specific binding
15 pair.

Specific Binding Molecule: A molecule that exhibits specific binding to its corresponding binding partner to form a specific binding pair. As used herein, this definition of specific binding molecule covers monoclonal and polyclonal
20 antibodies, antigen-binding fragments of these antibodies, hybrid antibodies, single-chain antibodies, and recombinant molecules capable of specific binding to a ligand.

Structural Gene: A DNA sequence that encodes through its template or messenger RNA ("mRNAII") a sequence of amino acids
25 characteristic of a specific polypeptide.

Transcription: Synthesis of RNA on a DNA template.

Translation: Synthesis of peptides on the mRNA template.

DESCRIPTION OF FIGURES

Fig. 1A depicts the core structure of a MAP protein with four arms, i.e., a 4-MAP protein; Fig. 1B depicts the core structure of a MAP protein with eight arms, i.e., an 8-MAP protein.

Fig.2 depicts data comparing the binding characteristics of several monoclonal antibodies to surface bound IgE (e.g., analogous to IgE expressed on the surface of B cells); IgE receptor (e.g., analogous to the Fc receptor on mast cells); and to a combination of IgE when bound by the IgE receptor. Thus each component assayed was immobilized on a solid surface.

Fig. 3 depicts the ability of recombinant exon 3 to inhibit conjugated antibody 8H.8 or conjugated antibody 15A.2 from binding to an IgE solid phase. Data for 8H.8 is depicted by speckled bar graphs, data for 15A.2 is depicted by solid black bar graphs.

Fig. 4 depicts the ability of soluble, purified canine IgE to inhibit monoclonal antibody 8H.8 (2.5 µg/ml) or monoclonal antibody 15A.2 (2.5 µg/ml) from binding to a canine IgE solid phase. Data for 8H.8 is depicted by speckled bar graphs; data for 15A.2 is depicted by solid black bar graphs.

Fig. 5 depicts the ability of monoclonal antibody 15A.2 or monoclonal antibody 8H.8 to inhibit IgE from binding to recombinant IgE receptor immobilized on a solid phase as detected by monoclonal antibody D9.

Fig. 6 depicts the amino acid sequences of the New England Biolabs PhDc7c phage display library that were bound

by the monoclonal antibody 15A.2. These sequences are shown in alignment with the protein sequence of canine IgE.

Fig. 7 depicts the alignment of the 15A.2 binding region from seven different mammals: dog, human, green monkey, cat, swine, mouse and horse.

Fig. 8 depicts the ability of phage displaying 15A.2 mimotope peptides to inhibit canine IgE from binding the 15A.2 monoclonal antibody on the solid phase.

Fig. 9 depicts the ability of a specific 15A.2 mimotope peptide sequence attached to the solid phase to be able to bind the 15A.2 mimotope monoclonal antibody.

Fig. 10A and 10B depict the ability of a 15A.2 mimotope peptide to be able to prevent the 15A.2 monoclonal antibody from binding canine IgE on the solid phase.

Fig. 11 depicts the ability of monoclonal antibody 8H.8 and monoclonal antibody 15A.2 to bind to purified canine IgE immobilized on a solid phase.

Fig. 12 depicts data from a study evaluating the ability of antibody 15A.2 conjugated to a signal moiety to bind to solid phases having various peptides immobilized thereon.

Fig. 13 depicts data from a study evaluating the ability of monoclonal antibody 14K2, known to bind with canine IgE exon 4, to bind with various peptides immobilized on a solid phase.

Fig. 14 depicts data from a study evaluating the ability of monoclonal antibody 15A.2, known to bind to canine IgE exon 3, to bind to various peptides immobilized on a solid phase.

Fig. 15 depicts data for studies that compared the binding of monoclonal antibody 8H.8, or a polyclonal antibody raised to recombinant IgE (Re-hu IgE), to bind to a solid

phase having peptide E3a.5 or substituted peptide E3a.5
(designated peptide S3a.5) immobilized thereon.

Fig. 16 depicts data from a study evaluating the ability
of antibody 8H.8 conjugated to a signal moiety to bind to
5 solid phases having various peptides immobilized thereon.

Fig. 17 depicts the ability of polyclonal anti-human IgE
antibodies to bind to various peptides immobilized on a solid
phase.

10 MODES FOR CARRYING OUT INVENTION

Proposed Mechanism of Action of Anti-IgE Monoclonal Antibodies in Causing Persistent Serum IgE Depletion

A hypothesis in accordance with the present invention is
that anti-IgE monoclonal antibodies affect IgE levels by
15 directly binding with the resident circulating IgE, after
which the bound complex is removed from the circulation.

Moreover, it is hypothesized that anti-IgE monoclonal
antibodies affect IgE levels by interfering with the
production of new IgE by B-cells. Memory B-cells, cells that
20 upon interacting with antigen and T-cell become antibody
producing cells (i.e., "memory IgE B-cells"), are involved in
replenishing serum IgE, and these cells contain IgE as their
cell surface receptors. Thus, it is possible that anti-IgE
monoclonal antibodies bind to the IgE on these antibody
25 producing cells and interfere with their function, such as by
down-regulation or by one or more mechanisms which lead to
cell destruction. Two general ways have been proposed for how

the binding of a monoclonal antibody to a memory B cell might interfere with production of IgE.

First, activation of a memory B cell, may also require the involvement of a surface IgE-binding factor designated
5 CD23. Binding of certain monoclonal antibodies to the cell surface IgE may preclude CD23 binding, and may thus lead to an inability to mount an IgE response.

Second, IgE antibody production by a memory IgE B cell may be reduced or eliminated by binding of a monoclonal
10 antibody that is directed against, or blocks, the receptor for the IgE molecule which is located on the surface of the memory B cell.

In the absence of a monoclonal antibody that leads to memory IgE B cell elimination or inactivation, however, it
15 would be expected that the memory IgE B-cells would be replenished, leading to the eventual replenishment of circulating IgE.

The serum levels of IgE are elevated in patients experiencing allergic disease. As disclosed herein, when
20 antibodies were generated that specifically bind to a species' IgE, and these antibodies were administered to a patient who is a member of the species ("passive immunization"), that patient's serum levels of IgE declined. As appreciated by one of ordinary skill in the art, the specific binding proteins in
25 accordance with the invention are administered in pharmacologically accepted dosages, and can be administered with suitable biocompatible diluents. Also disclosed herein are a method and related compositions that serve to induce a response to a self-peptide, such as an IgE molecule, whereby
30 the immune system is manipulated so as to allow an

auto-reactive B cell to become an antibody-secreting B cell ("active immunization").

Receptors are present on mast cells that bind to IgE from the circulation. When IgE has become bound to the receptors on mast cells, the IgE molecules can become crosslinked. Upon cross-linking these IgE molecules, the mast cell is induced to release histamine. Histamine is an agent that induces the manifestation of allergic symptoms. Generally, cross-linking occurs by binding of the IgE molecules to an antigen, e.g., an allergen. However, a monoclonal antibody that binds to IgE could serve as a means for cross-linking IgE which has become bound to the surface of mast cells.

For allergic individuals, if anti-IgE antibodies were present in circulation, it becomes easy to envisage that the binding of such auto-anti-IgE to IgE molecules on mast cells could serve to cross-link the IgE molecules on those cells and exacerbate an ongoing allergic response even in the absence of allergen. This occurrence is clearly undesirable in the context of allergy treatment. An antibody that achieved this function would therefore not be preferred for use as a therapeutic in accordance with the invention. Therefore, an antibody that achieves such crosslinking is disadvantageous, and is not preferred in accordance with the invention.

Two approaches exist for producing monoclonal antibodies which target IgE, but which do not cross-link IgE molecules which have become bound to a mast cell. The first is to produce monoclonal antibodies that are directed to an epitope of an IgE molecule that is only accessible when the IgE is in circulation. Since the monoclonal antibody can only bind to

IgE when it is in circulation, it will not be able to bind to IgE that has become bound to the surface of a mast cell.

An alternative approach for producing anti-IgE antibodies has been hypothesized by Stanworth et al, e.g., U.S. Patent No. 5,601,821 issued 11 February 1997. The antibodies of Stanworth are disclosed to cross-link IgE on mast cells, but in a manner that does not induce histamine release. Stanworth has disclosed an epitope of the human IgE molecule which must be available or accessible after IgE molecules have become cross-linked on the surface of a mast cell, in order for the mast cell to release histamine. Thus, Stanworth disclosed an antibody that binds to that particular epitope. Accordingly, monoclonal antibodies that are directed to this IgE epitope will serve to cross-link the IgE, but will not permit the mast cell to release histamine.

The approach followed in accordance with the present invention is the development of antibodies, either *ex vivo* monoclonal or *in vivo* anti-self antibodies, directed to epitopes which are accessible on circulating IgE but which are not accessible when such IgE becomes bound to a mast cell.

Passive Immunity

Monoclonal Antibodies 15A.2 and 8H.8

Monoclonal antibody 15A.2 binds to canine IgE. The 15A.2 monoclonal antibody was derived by immunizing mice with affinity-purified canine IgE. The epitope bound by 15A.2 is conformational, not linear. As indicated by data depicted in Figure 2, 15A.2 did not bind to the IgE receptor; nor did it bind to IgE when bound to receptor. However, 15A.2 exhibited

affinity for free IgE. It binds to recombinant canine exon 3 fusion proteins in an enzyme linked immunosorbent assay (ELISA) and by Western blot, but not to other recombinant canine IgE fusion proteins.

5 Characterization experiments of the 15A.2 monoclonal antibody showed that 15A.2 did not bind to IgE that was already bound by the IgE receptor on mast cells. Thus, it appeared that access to the epitope bound by 15A.2 was hindered by IgE binding to the Fc receptor on mast cells.
10 Accordingly, 15A.2 will not crosslink IgE bound to mast cells. This finding was demonstrated by binding studies with a recombinant receptor discussed herein.

Monoclonal antibody 8H.8 also binds to canine IgE. The 8H.8 monoclonal antibody was derived by immunizing mice with a
15 shortened version of exon 3 of the canine IgE molecule, designated exon 3a. Exon 3a contains the C-terminal 71 amino acids of the full length exon 3. Previous studies (data not presented herein) had shown that immunizing mice with the full length exon 3 did not generate antibodies having specificity
20 such as that ultimately found with the 8H.8 antibody.

Characterization experiments of the 8H.8 monoclonal antibody showed that, unlike monoclonal antibodies derived by immunization with all other recombinant IgE sequences, 8H.8 would also bind to native canine IgE. Additionally, like
25 15A.2 and as depicted in Figure 2, it was found that 8H.8 did not bind to IgE that was already bound by the IgE receptor on mast cells. Thus, it appeared that access to the epitope bound by 8H.8 was also hindered by IgE binding to the Fc receptor on mast cells. As a result, 8H.8 will not crosslink

IgE bound to mast cells. This finding was demonstrated by binding studies with a recombinant receptor discussed herein.

Competitive assays were performed between an antibody, 8H.8 or 15A.2, and soluble exon 3 to identify the level of inhibition of binding of these antibodies to affinity purified native IgE immobilized on the solid phase of an ELISA. The results from this study are depicted in Figure 3. In Figure 3 it is seen that increasing the concentration of recombinant exon 3 inhibited the binding of monoclonal antibody 8H.8, but did not inhibit antibody 15A.2.

In addition, the ability of these monoclonal antibodies to inhibit IgE from binding to recombinant IgE receptor on an ELISA solid phase was examined.

Additionally, competitive assays were performed with antibody 8H.8, and analogous assays were performed with 15A.2, where the antibody was in competition with affinity-purified canine IgE in solution and IgE on an ELISA solid phase. Data from these assays is depicted in Figure 4.

These studies suggested that 15A.2 had a high affinity for soluble native canine IgE because as the concentration of the soluble IgE was increased, the binding of 15A.2 to the immobilized immunoglobulin dropped appreciably, indicating that 15A.2 was now binding to the soluble IgE. In contrast, it is seen that antibody 8H.8 has a much lower affinity for soluble IgE, because increasingly high concentrations of the soluble IgE did not inhibit the ability of 8H.8 to bind to the immobilized IgE. Thus, the data suggest that soluble IgE was not effective at inhibiting 8H.8 from binding to IgE immobilized on a solid phase. Thus, the affinity of 8H.8 for

soluble canine IgE was lower than the affinity of this monoclonal antibody for IgE on a solid phase.

These studies further suggested that 8H.8 had a low affinity for soluble native canine IgE, because increasingly
5 high concentrations of the soluble IgE did not inhibit the ability of 8H.8 to bind to the immobilized IgE. In contrast, it is seen that antibody 15A.2 has a much higher affinity for soluble IgE, since as the concentration of the soluble IgE was increased, the binding of 15A.2 to the immobilized
10 immunoglobulin dropped appreciably, indicating that 15A.2 was now binding to the soluble IgE. Thus, the data suggested that soluble IgE was not effective at inhibiting 8H.8 from binding to IgE immobilized on a solid phase. Thus, the affinity of 8H.8 for soluble canine IgE was lower than the affinity of
15 this monoclonal for IgE on a solid phase.

To further substantiate the finding that 15A.2 has a higher affinity for soluble native IgE, and that 8H.8 had a low affinity for soluble native IgE, studies were performed where 8H.8 was used, and analogous studies were conducted with
20 15A.2, to determine the extent to which each antibody inhibits binding of the soluble IgE to a recombinant IgE receptor solid phase. A labeled antibody D9 was used as a means for detection in the studies depicted in Figure 5. The monoclonal antibody D9 is known to bind to IgE when bound to receptor.
25 For the studies depicted in Figure 5, the native IgE was present at a concentration of 2.5 micrograms per milliliter. As shown in Figure 5, 15A.2 was effective at inhibiting binding of the native IgE to recombinant IgE receptor at antibody concentrations as low as 190 nanograms per
30 milliliter. In contrast, 3,125 nanograms per milliliter of

8H.8 were necessary to inhibit the binding of native IgE to the recombinant IgE receptor. Thus, since more 8H.8 than 15A.2 was required to inhibit the binding of native IgE to the recombinant receptor, it was seen that 8H.8 had a lower
5 affinity for the soluble IgE. It appears, therefore, that when a sufficient concentration of either 8H.8 or 15A.2 antibodies is provided, that the antibodies impair the binding of soluble IgE to the IgE receptor.

Furthermore, as indicated by the data depicted in Figure
10 2, the avidity of 8H.8 binding for native canine IgE was greatly increased when the IgE was immobilized. These studies suggested that 8H.8 had a low affinity for soluble native canine IgE, but that when the IgE is immobilized on a surface, or when it is expressed on the surface of a memory B cell, the
15 avidity of 8H.8 binding for native canine IgE was greatly increased.

Accordingly, in view of these findings, it was hypothesized that the region within native IgE recognized by 8H.8 might be partially hidden, particularly when the IgE is
20 in serum. A partially sequestered amino acid sequence of IgE may not be readily exposed to the native canine immune system and its normal protective mechanisms. Furthermore, since the full length exon 3 did not lead to the generation of antibodies having 8H.8-like specificity, the full length
25 sequence might contain suppressor peptides capable of down-regulating or eliminating an auto-anti-IgE response which is specific against the epitope recognized by the 8H.8 antibody. Either mechanism could serve to avoid production of an autoimmune response to self-antigens.

The implications of these findings are that 8H.8 antibody, when used as an allergy therapeutic in dogs, would preferentially bind to IgE on memory B cells rather than to IgE in solution. Moreover, 8H.8 has a low binding affinity for IgE when bound by Fc receptor on mast cells.

Phage display technology was used to map the epitope bound by the 8H.8 antibody. A preferred method for performing phage display technology is accomplished by use of a Ph.D. Phage Display Peptide Library Kit (New England BioLabs, Beverly Mass.). It was found that a 7 amino acid peptide (Thr-Leu-Leu-Glu-Tyr-Arg-Met) inhibited monoclonal antibody 8H.8 from competitive binding to either native or recombinant canine IgE. This sequence contained 6 amino acids common in form and/or spacing to the C-terminal region of exon 3. An 11 amino acid peptide synthesized from this region (Gly-Met-Asn-Leu-Thr-Trp-Tyr-Arg-Glu-Ser-Lys) designated E3a.5 also inhibited monoclonal antibody 8H.8 from competitive binding to native or recombinant IgE.

It is believed that antibody responses with specificity such as that of 8H.8 do not occur naturally, even upon occurrence of events which would induce auto-anti-IgE responses to other epitopes, because the 8H.8 epitope is only partially available for recognition. Nevertheless, it is hypothesized that antibodies to an 8H.8-type epitope, generated from peptide immunization in accordance with the invention do bind to the partially available epitope, as does the 8H.8 monoclonal antibody.

It is further hypothesized that the region within native IgE recognized by 15A.2 might serve as an immunogen to induce auto-anti-IgE responses and might generate antibodies capable

of binding to IgE+ B cells and of down regulating IgE synthesis. The implications of these findings are that 15A.2 antibody, when used as an allergy therapeutic in dogs, would prevent IgE from binding to mast cells and potentially affect the synthesis of IgE by B cells.

Phage display technology was used to map the epitope bound by the 15A.2 antibody. As noted above, a preferred method for performing phage display technology is accomplished by use of a Ph.D. Phage Display Peptide Library Kit (New England BioLabs, Beverly Mass.). Figure 6 depicts the amino acid sequences of the PhDc7c library that were bound by the monoclonal antibody 15A.2. These sequences are shown in alignment with the protein sequence of canine IgE. Figure 7 depicts the alignment of the 15A.2 epitope from seven different mammals: dog, human, green monkey, cat, swine, mouse and horse.

Figure 8 portrays the ability of different phage displaying 15A.2 mimotope peptides to inhibit canine IgE from binding the 15A.2 monoclonal antibody on the solid phase. 466 μ l of biotinylated 15A.2 antibody (10 μ g/ml) were mixed with 466 μ l of phage from a fresh overnight culture. Into each well of a microtiter plate 100 μ l of the mixture was added. The antibodies and phage were allowed to bind for 2 ½ hours. The wells were then coated with strepavidin. The plates were washed three times with standard wash buffer to remove loosely bound material. A serial dilution of canine IgE was prepared, starting with the concentration of 1 μ g/100 μ l of PBS, 0.1% Tween. 100 μ l of dilution IgE was added per well and allowed to bind for 10 minutes. The competition reaction was stopped

by washing the plates five times with wash solution. The plate was then developed with HRPO linked D9 monoclonal antibody, which binds to domain 4 of IgE, to visualize the IgE in the solid phase. Loss of signal indicates the phage successfully repelled canine IgE competition. Table 1 shows the concentrations of the reactants at the various points on the x-axis of figure 8.

TABLE 1

	IgE	phage
1	1.0 μ g	0
2	1.0 μ g	50 μ l
3	0.5 μ g	50 μ l
4	0.25 μ g	50 μ l
5	0.125 μ g	50 μ l

10

Using the New England Biolabs PhD12 phage display library, it was found that a 12 amino acid peptide (SEQ ID NO:11 Val-Thr-Leu-Cys-Pro-Asn-Pro-His-Ile-Pro-Met-Cys) inhibited monoclonal antibody 15A.2 from competitive binding to either native or recombinant canine IgE. This sequence contained 4 amino acids common in form and/or spacing to the N-terminal region of exon 3.

Figure 9 displays the ability of 15A.2 monoclonal antibody to bind a synthetic peptide. The peptide SEQ ID NO:3 Ser-Val-Thr-Leu-Cys-Pro-Asn-Pro-His-Ile-Pro-Met-Cys-Gly-Gly-Gly-Lys was synthesized and biotinylated on the epsilon carbon of the Lys residue. This sequence corresponds to the isolate M13-48. The peptide was treated in a manner to promote reduction and cyclization of the cysteines to form a cyclic peptide. A serial dilution of the peptide was prepared and

25

bound to strepavidin-coated microtiter plates (5 µg strepavidin per well). Bound peptide was detected with HRPO conjugated 15A.2 monoclonal antibody. Figure 9 shows the results of this experiment. This solid phase specific 15A.2
5 mimotope peptide bound the 15A.2 monoclonal antibody in a concentration dependent manner.

Figures 10A and 10B depict the ability of a 15A.2 mimotope peptide to prevent the 15A.2 monoclonal antibody from binding canine IgE on the solid phase. Plates were coated
10 with canine IgE at a concentration of 1 µg/ml. A serial dilution of the synthetic peptide was added to HRPO conjugated 15A.2 monoclonal antibody (final concentration 1 µg/ml) and allowed to bind for two hours. 100 µl of the 15A.2/peptide mixture was added to the wells and allowed to bind 1 hour.
15 The reaction was stopped by washing the plates six times with wash buffer. The plates were developed with HRPO substrate, the reactions stopped with stop mix, and the plates were measured for O.D. using a plate reader.

Apart from active immunization with antibody 15A.2 or
20 8H.8 to induce auto-anti-IgE production *in vivo*, this invention also comprises a specific binding molecule that specifically binds a ligand of the type bound by 15A.2 or 8H.8, as well as the therapeutic or prophylactic use thereof. Thus, the invention comprises a monoclonal antibody specific
25 for a conservative variant of a sequence bound by 15A.2, for a conservative variant of a sequence bound by 8H.8, or for a native sequence of canine IgE up to 100 amino acids from the C' terminal portion of exon 3. The specific binding molecules of the invention can be used in a method in accordance with

the invention as a treatment for or as prophylaxis for allergy symptoms, i.e., passive immunization.

Accordingly, administration to a dog of either an antibody in accordance with the invention, such as 15A.2 or 8H.8, or a peptide in accordance with the invention, such as the 7, 11 or 12 amino acid peptide, leads to a diminution of further IgE production. This may occur, for example, by the 15A.2 or 8H.8 antibody binding to the memory B cell and preventing further IgE production. For a peptide, its administration would lead to production of antibodies of comparable specificity and effect as 15A.2 or 8H.8.

EXAMPLES

Example 1

Figure 11 depicts results of the binding study where antibody 8H.8 and antibody 15A.2 were separately assayed to determine their ability to bind to a solid phase having affinity purified canine IgE immobilized thereon. These results showed that those 8H.8 and 15A.2 bind to native IgE when immobilized on a solid phase. The results from these studies suggest that the binding of each of these antibodies to the surface bound IgE was of comparable affinity. To determine whether the affinity of binding of each antibody was, in fact, comparable, further studies were performed.

Figure 12 depicts results of the study where antibody 8H.8 conjugated to a signal moiety was reacted at various concentration with solid phases having various peptides immobilized thereon. The data depicted by (◆) reflects a solid phase having canine IgE immobilized thereon; data depicted by (■) depicts solid phase having E3a.5 immobilized

thereon; data depicted by (π) depicts solid phase have E3a.5 extended peptide immobilized thereon, data depicted by an (X) reflects data for a solid phase having an E3a.5 scrambled peptide immobilized thereon, data depicted by (*) depicts data for solid phase having the seven amino acid peptide identified by phage display technology to which SH8 binds immobilized thereon. Thus, it is seen that 8H.8 binds to each solid phase with the exception solid phase having the E3a.5 scrambled peptide.

Monoclonal antibody 14K2 is known to bind exclusive to canine IgE exon 4. This monoclonal antibody was reacted with various solid phases having different peptides immobilized thereon. The data depicted by (\blacklozenge) reflects a solid phase having canine IgE immobilized thereon; data depicted by (\blacksquare) depicts solid phase having E3a.5 immobilized thereon; data depicted by (π) depicts solid phase have E3a.5 extended peptide immobilized thereon; data depicted by an (X) reflects data for a solid phase having an E3a.5 scrambled peptide immobilized thereon; and data depicted by (*) depicts data for solid phase having the seven amino acid peptide identified by phage display technology to which 8H.8 binds immobilized thereon. Thus, it is seen that 14K2 only binds to a solid phase having the entire canine IgE molecule immobilized thereon. This data is depicted in Figure 13.

Monoclonal antibody 15A.2 is believed to bind only to canine IgE exon 3. Figure 14 depicts data for studies which evaluated the binding of 15A.2 to various peptides. The data depicted by (\blacklozenge) reflects a solid phase having canine IgE immobilized thereon; data depicted by (\blacksquare) depicts solid phase

having E3a.5 immobilized thereon; data depicted by (π) depicts solid phase have E3a.5 extended peptide immobilized thereon, data depicted by an (X) reflects data for a solid phase having an E3a.5 scrambled peptide immobilized thereon, data depicted
5 by (*) depicts data for solid phase having the seven amino acid peptide identified by phage display technology to which 8H.8 binds immobilized thereon. These binding studies showed that 15A.2 bound only to a solid phase which had the entire canine IgE molecule immobilized thereon. This data indicated
10 that 15A.2 does not bind the same epitope as that bound by 8H.8.

Figure 15 depicts data for studies that compare the binding of 8H.8 or polyclonal antibodies raised to recombinant human IgE, to peptide E3a.5 or to peptide E3a.5 with an amino
15 acid substitution making the peptide more analogous to a sequence in the human genome which encodes human IgE, designated peptide S3a.5. In Figure 15 data depicted by the (\blacklozenge) reflects substituted 3a.5 compared with 8H.8; data depicted by (\blacksquare) reflects substituted peptide 3a.5 and Re-Hu
20 IgE: data depicted by (π) reflects peptide E3a.5 and 8H.8 and, data depicted by and (X) reflects data regarding peptide E3a.5 and polyclonal Re Hu IgE. These data indicated that only 8H.8 became bound to E3a.5 on a solid phase, no other combination exhibited binding.

25 Figure 16 depicts the data on studies that compared the binding of 8H.8 to various peptides immobilized on a solid phase. Data depicted by (\blacklozenge) reflects data for a solid phase having canine IgE immobilized thereon; data depicted by (\blacksquare) reflects data for a solid phase having a peptide E3a.5

extended immobilized thereon; data depicted by (π) reflects data for a solid phase having human IgE immobilized thereon and, data depicted by an (X) reflects data for a solid phase having a 8H.8 peptide immobilized thereon. Accordingly it is
5 seen that 8H.8 binds to canine IgE, peptide E3a.5 extended or the seven amino acid peptide identified by phage display technology as the binding epitope for 8H.8, when each of these peptides were immobilized on a solid phase. Additionally, it is seen that 8H.8 does not bind to human IgE.

10 Figure 17 depicts data for studies which compare the ability of polyclonal anti human IgE antibodies ability to bind to solid phases having various peptides immobilized thereon. Data depicted by (\blacklozenge) reflects data for a solid phase having canine IgE immobilized thereon; data depicted by (\blacksquare)
15 reflects data for a solid phase having a peptide E3a.5 extended immobilized thereon; data depicted by (π) reflects data for a solid phase having human IgE immobilized thereon and, data depicted by an (X) reflects data for a solid phase having a 8H.8 peptide immobilized thereon. Thus, it was seen
20 that the anti-human IgE polyclonal antibodies bound to human IgE when immobilized on a solid phase. It was also seen that the polyclonal antiserum exhibited limited binding to peptide E3a.5 extended when the polyclonal antibodies were present at very high concentrations; this was believed to be a binding
25 artifact due to the high antibody concentration. Thus, it was found that antibodies to Human IgE do not specifically bind to canine IgE or to peptides in accordance with the invention.

Example 2

The amino acid sequences for the epitope bound by 8H.8, the 11 amino acid sequence used in the immunization studies presented herein, as well as analogous sequences from feline and Human IgE sequences are set forth in Table 2. Additionally, a substituted canine sequence was prepared having an amino acid substitution which made the peptide more closely analogous to the human peptide.

TABLE 2		
Species/Source	Sequence	SEQ ID NO.
mimotope bound by 8H.8	T-L-L-E-Y-R-M	9
canine	G-M-N-L-T-W-Y-R-E-S-K	10
human	V-N-L-T-W-S-R	15
feline	G-M-T-L-T-W-S-R-E-N-G	16
Substituted Canine Sequence	G-M-N-L-T-W-S-R-E-S-K	17

Each of the peptides in Table 2 was placed on a solid phase and studies were performed to identify if 8H.8 would bind to the surface-bound peptide. It was found that 8H.8 bound to a surface having the mimotope peptide thereon, and to a surface having the 11 amino acid peptide bound thereon. It was found that 8H.8 did not bind to the human peptide, the feline peptide or to the canine sequence which had an amino acid substitution which made it more analogous to the human sequence, when either of these peptides were surface bound.

20 Closing

As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example,

5 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar to or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described. All publications mentioned
10 herein are fully incorporated herein by reference.

WHAT IS CLAIMED IS:

1. A specific binding protein which specifically binds to native canine free or B cell-bound IgE, and which does not bind to IgE when the IgE is bound to a receptor on a mast
5 cell.

2. The specific binding protein of claim 1 wherein the B cell-bound IgE is IgE expressed on the surface of a canine B
10 cell.

3. A method for treatment or prophylaxis for canine allergy, said method comprising:
providing a specific binding protein of claim 1;
and,
15 administering the provided binding protein to a dog.

4. The method of claim 3 further comprising a step of mixing the provided binding protein with a diluent prior to the administering step, and wherein the administering step
20 comprises administering the mixture of the binding protein and the diluent.

5. The method of claim 3 further comprising a step of administering a booster dose of the provided binding protein,
25 following the administering step.

6. A specific binding protein which specifically binds to an isolated and purified peptide comprising a leucine

positioned two peptide bonds away from a tyrosine-arginine pair.

7. The specific binding protein of claim 6 which
5 specifically binds to peptide comprising the form:
leucine-blank-blank-tyrosine-arginine, tyrosine-arginine-
blank-blank-leucine, or, leucine-blank-blank-tyrosine-
arginine-blank-blank-leucine, where blank is any amino acid.

10 8. The specific binding protein of claim 7 wherein at
least one blank is an amino acid with an aromatic ring.

9. A specific binding protein of claim 6 which is an
antibody.

15 10. The specific binding protein of claim 9 which is a
monoclonal antibody.

11. An antibody of claim 9 which is raised to an
20 isolated and purified peptide comprising a leucine positioned
two peptide bonds away from a tyrosine-arginine pair, and
wherein the peptide consists of from 5 to 71 amino acids.

12. A method for treatment or prophylaxis for canine
25 allergy, said method comprising:

providing a specific binding protein in accordance
with claim 8; and,

administering the provided binding protein to a dog.

13. The method of claim 12 further comprising a step of
mixing the provided binding protein with a diluent prior to
the administering step, and wherein the administering step
comprises administering the mixture of the binding protein and
5 the diluent.

14. The method of claim 12 further comprising a step of
administering a booster dose of the provided binding protein,
following the administering step.

15. An antibody which is raised to an isolated and
purified peptide comprising an amino acid sequence which
comprises Thr-Leu-Leu-Glu-Tyr-Arg-Met, or a conservative
variant thereof.

16. The antibody of claim 15 that binds to a defined
epitope.

17. A recombinant binding molecule which specifically
20 binds to the defined epitope bound by the antibody of claim
16.

18. A method for treatment or prophylaxis for canine
allergy, said method comprising:
25 providing an antibody in accordance with claim 15;
and,
administering the provided binding protein to a dog.

19. The method of claim 18 further comprising a step of
30 mixing the provided antibody with a diluent prior to the

administering step, and wherein the administering step comprises administering the mixture of the antibody and the diluent.

5 20. The method of claim 18 further comprising a step of administering a booster dose of the provided antibody, following the administering step.

10 21. An antibody which is raised to an isolated and purified peptide comprising an amino acid sequence which comprises Gly-Met-Asn-Leu-Thr-Trp-Tyr-Arg-Glu-Ser-Lys, or a conservative variant thereof.

15 22. The antibody of claim 21 that binds to a defined epitope.

20 23. A recombinant binding molecule which specifically binds to the defined epitope bound by the antibody of claim 22.

24. A method for treatment or prophylaxis for canine allergy, said method comprising:
 providing an antibody in accordance with claim 21;
and,
25 administering the provided binding protein to a dog.

25. The method of claim 24 further comprising a step of mixing the provided antibody with a diluent prior to the administering step, and wherein the administering step

comprises administering the mixture of the antibody and the diluent.

26. The method of claim 24 further comprising a step of
5 administering a booster dose of the provided antibody,
following the administering step.

27. A specific binding protein which is raised to a
multiply antigenic peptide comprising multiple copies of an
10 isolated and purified peptide which comprises a leucine
positioned two peptide bonds away from a tyrosine-arginine
pair.

28. The specific binding protein of claim 27 which is
15 raised to a multiply antigenic peptide which comprises
multiple copies of the isolated and purified peptide
comprising a leucine positioned two peptide bonds away from a
tyrosine-arginine pair, and wherein the peptide consists of
from 5 to 71 amino acids.

20

29. The specific binding protein of claim 27 that
specifically binds to a defined epitope.

30. A recombinant binding molecule which specifically
25 binds to the defined epitope bound by the binding protein of
claim 29.

31. A method for treatment or prophylaxis for canine
allergy, said method comprising:

providing a specific binding protein of claim 27;

and,

administering the provided binding protein to a dog.

5 32. The method of claim 31 further comprising a step of
mixing the provided binding protein with a diluent prior to
the administering step, and wherein the administering step
comprises administering the mixture of the binding protein and
the diluent.

10

33. The method of claim 31 further comprising a step of
administering a booster dose of the provided binding protein,
following the administering step.

15

34. A specific binding protein which is raised to a
recombinant plant virus particle comprising at least one copy
of an isolated and purified peptide comprising a leucine
positioned two peptide bonds away from a tyrosine-arginine
pair.

20

35. The specific binding protein of claim 34 which is
raised to the recombinant plant virus particle, wherein the
particle comprises at least one copy of the isolated and
purified peptide comprising a leucine positioned two peptide
25 bonds away from a tyrosine-arginine pair, and wherein the
peptide consists of from 5 to 71 amino acids.

30

37. A recombinant binding molecule which specifically binds to the defined epitope bound by the binding protein of claim 36.

5 38. A method for treatment or prophylaxis for canine allergy, said method comprising:

providing a specific binding protein in accordance with claim 34; and,

administering the provided binding protein to a dog.

10

39. The method of claim 38 further comprising a step of mixing the provided binding protein with a diluent prior to the administering step, and wherein the administering step comprises administering the mixture of the binding protein and
15 the diluent.

40. The method of claim 39 further comprising a step of administering a booster dose of the provided binding protein, following the administering step.

20

41. A monoclonal antibody which is 8H.8.

42. The monoclonal antibody of claim 41 that specifically binds to a defined epitope.

25

43. A recombinant binding molecule which specifically binds to the defined epitope bound by the antibody of claim 41.

44. A method for treatment or prophylaxis for canine allergy, said method comprising:

providing the monoclonal antibody of claim 41 and,
administering the provided monoclonal antibody to a
5 dog.

45. The method of claim 44 further comprising a step of mixing the provided monoclonal antibody with a diluent prior to the administering step, and wherein the administering step
10 comprises administering the mixture of the monoclonal antibody and the diluent.

46. The method of claim 44 further comprising a step of administering a booster dose of the provided monoclonal
15 antibody, following the administering step.

47. A specific binding protein which specifically binds to an isolated and purified peptide comprising cysteine-blank-proline-histidine-blank-proline-blank-blank-cysteine, where
20 blank is any amino acid.

48. The specific binding protein of claim 47 which is an antibody.

25 49. The specific binding protein of claim 48 which is a monoclonal antibody.

50. An antibody of claim 48 which is raised to an isolated and purified peptide comprising cysteine-blank-

proline-histidine-blank-proline-blank-blank-cysteine, where
blank is any amino acid.

51. A method for treatment or prophylaxis for canine
5 allergy, said method comprising:
 providing a specific binding protein in accordance
with claim 47; and,
 administering the provided binding protein to a dog.

10 52. The method of claim 51 further comprising a step of
mixing the provided binding protein with a diluent prior to
the administering step, and wherein the administering step
comprises administering the mixture of the binding protein and
the diluent.

15 53. The method of claim 51 further comprising a step of
administering a booster dose of the provided binding protein,
following the administering step.

20 54. An antibody which is raised to an isolated and
purified peptide comprising an amino acid sequence which
comprises Cys-Pro-Asn-Pro-His-Ile-Pro-Met-Cys, or a
conservative variant thereof.

25 55. The antibody of claim 54 wherein the amino acid
sequence comprises Ser-Val-Thr-Leu-Cys-Pro-Asn-Pro-His-
Ile-Pro-Met-Cys-Gly-Gly-Gly.

30 56. The antibody of claim 54 that binds to a defined
epitope.

57. A recombinant binding molecule which specifically binds to the defined epitope bound by the antibody of claim 56.

5

58. A method for treatment or prophylaxis for canine allergy, said method comprising:

providing an antibody in accordance with claim 54;
and,

10 administering the provided binding protein to a dog.

59. The method of claim 58 further comprising a step of mixing the provided antibody with a diluent prior to the administering step, and wherein the administering step
15 comprises administering the mixture of the antibody and the diluent.

60. The method of claim 58 further comprising a step of administering a booster dose of the provided antibody,
20 following the administering step.

61. A specific binding protein which specifically binds to an isolated and purified peptide comprising cysteine-blank-blank-proline-histidine-blank-blank-blank-cysteine, where
25 blank is any amino acid.

62. The specific binding protein of claim 61 which is an antibody.

63. The specific binding protein of claim 62 which is a monoclonal antibody.

64. An antibody of claim 62 which is raised to an
5 isolated and purified peptide comprising cysteine-blank-blank-
proline-histidine-blank-blank-blank-cysteine, where blank is
any amino acid.

65. A method for treatment or prophylaxis for canine
10 allergy, said method comprising:
 providing a specific binding protein in accordance
 with claim 61; and,
 administering the provided binding protein to a dog.

66. The method of claim 65 further comprising a step of
15 mixing the provided binding protein with a diluent prior to
the administering step, and wherein the administering step
comprises administering the mixture of the binding protein and
the diluent.

20

67. The method of claim 65 further comprising a step of
administering a booster dose of the provided binding protein,
following the administering step.

25 68. An antibody which is raised to an isolated and
purified peptide comprising an amino acid sequence which
comprises Cys-Pro-Asn-Pro-His-Ile-Pro-Met-Cys or a
conservative variant thereof.

69. The antibody of claim 68 wherein the amino acid sequence comprises Ser-Val-Thr-Leu-Cys-Pro-Asn-Pro-His-Ile-Pro-Met-Cys-Gly-Gly-Gly.

5 70. The antibody of claim 68 that binds to a defined epitope.

71. A recombinant binding molecule which specifically binds to the defined epitope bound by the antibody of claim
10 70.

72. A method for treatment or prophylaxis for canine allergy, said method comprising:
 providing an antibody in accordance with claim 68;
15 and,
 administering the provided binding protein to a dog.

73. The method of claim 72 further comprising a step of mixing the provided antibody with a diluent prior to the
20 administering step, and wherein the administering step comprises administering the mixture of the antibody and the diluent.

74. The method of claim 72 further comprising a step of
25 administering a booster dose of the provided antibody, following the administering step.

75. An antibody which is raised to an isolated and purified peptide comprising an amino acid sequence which

comprises Cys-Pro-Asn-Pro-His-Asn-Pro-Tyr-Cys or a conservative variant thereof.

76. The antibody of claim 75 wherein the amino acid
5 sequence comprises Ser-Ala-Cys-Pro-Asn-Pro-His-Asn-Pro-Tyr-Cys-Gly-Gly-Gly.

77. The antibody of claim 75 that binds to a defined epitope.

10

78. A recombinant binding molecule which specifically binds to the defined epitope bound by the antibody of claim 77.

79. A method for treatment or prophylaxis for canine allergy, said method comprising:
providing an antibody in accordance with claim 76;
and,
administering the provided binding protein to a dog.

20

80. The method of claim 79 further comprising a step of mixing the provided antibody with a diluent prior to the administering step, and wherein the administering step comprises administering the mixture of the antibody and the
25 diluent.

81. The method of claim 79 further comprising a step of administering a booster dose of the provided antibody, following the administering step.

30

82. A monoclonal antibody which is 15A.2.

83. The monoclonal antibody of claim 82 that specifically binds to a defined epitope.

5

84. A recombinant binding molecule which specifically binds to the defined epitope bound by the antibody of claim 83.

10

85. A method for treatment or prophylaxis for canine allergy, said method comprising:

providing the monoclonal antibody of claim 82 and,
administering the provided monoclonal antibody to a dog.

15

86. The method of claim 85 further comprising a step of mixing the provided monoclonal antibody with a diluent prior to the administering step, and wherein the administering step comprises administering the mixture of the monoclonal antibody and the diluent.

20

87. The method of claim 85 further comprising a step of administering a booster dose of the provided monoclonal antibody, following the administering step.

25

ABSTRACT

Disclosed are binding proteins that specifically bind to an isolated and purified peptide comprising a leucine positioned two peptide bonds away from a tyrosine-arginine pair. Other binding proteins of the invention include those
5 that specifically bind to an isolated and purified peptide with the sequence cysteine-blank-blank-proline-histidine-blank-blank-blank-cysteine or cysteine-blank-proline-histidine-blank-proline-blank-blank-cysteine.

FIGURE 1

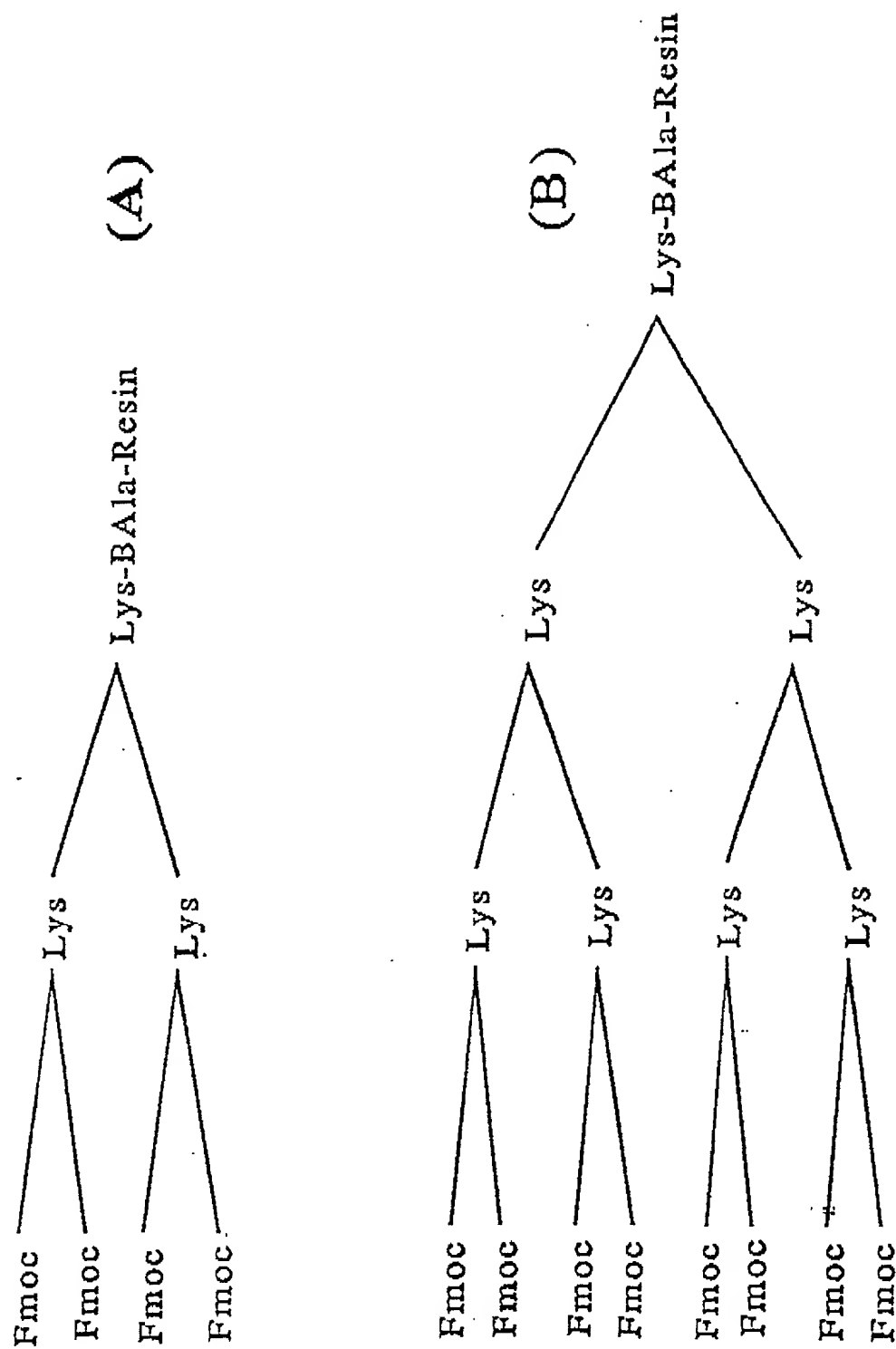
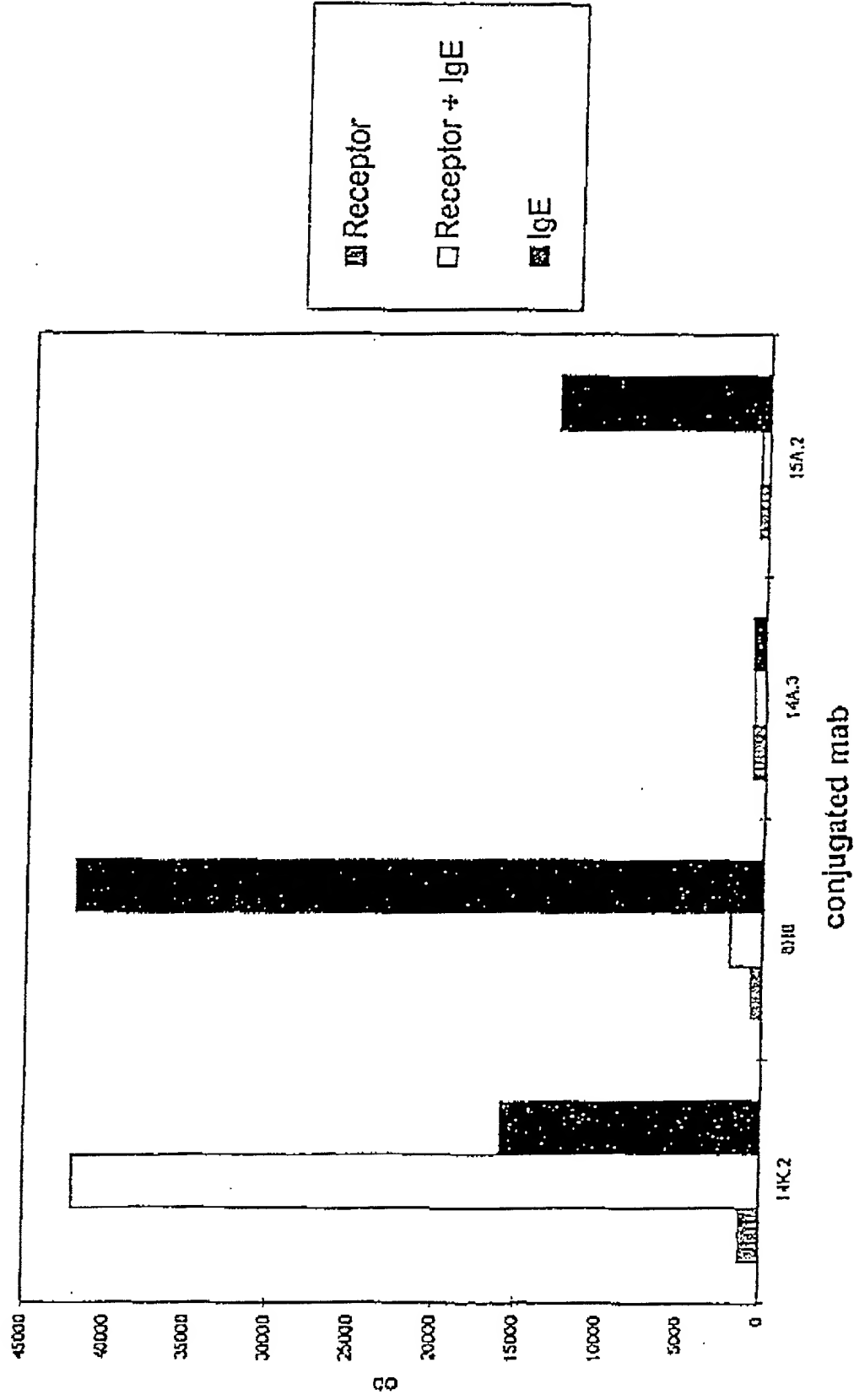


FIGURE 2

Anti-IgE Mab binding to IgE or receptor bound IgE



bioRxiv preprint doi: <https://doi.org/10.1101/092750>; this version posted October 1, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

FIGURE 3

Ability of Recombinant Exon 3 to Inhibit Conjugated 8H8 (5 ug/ml) or 15A.2 (2.5 ug/ml) Mab from binding to an IgE solid phase

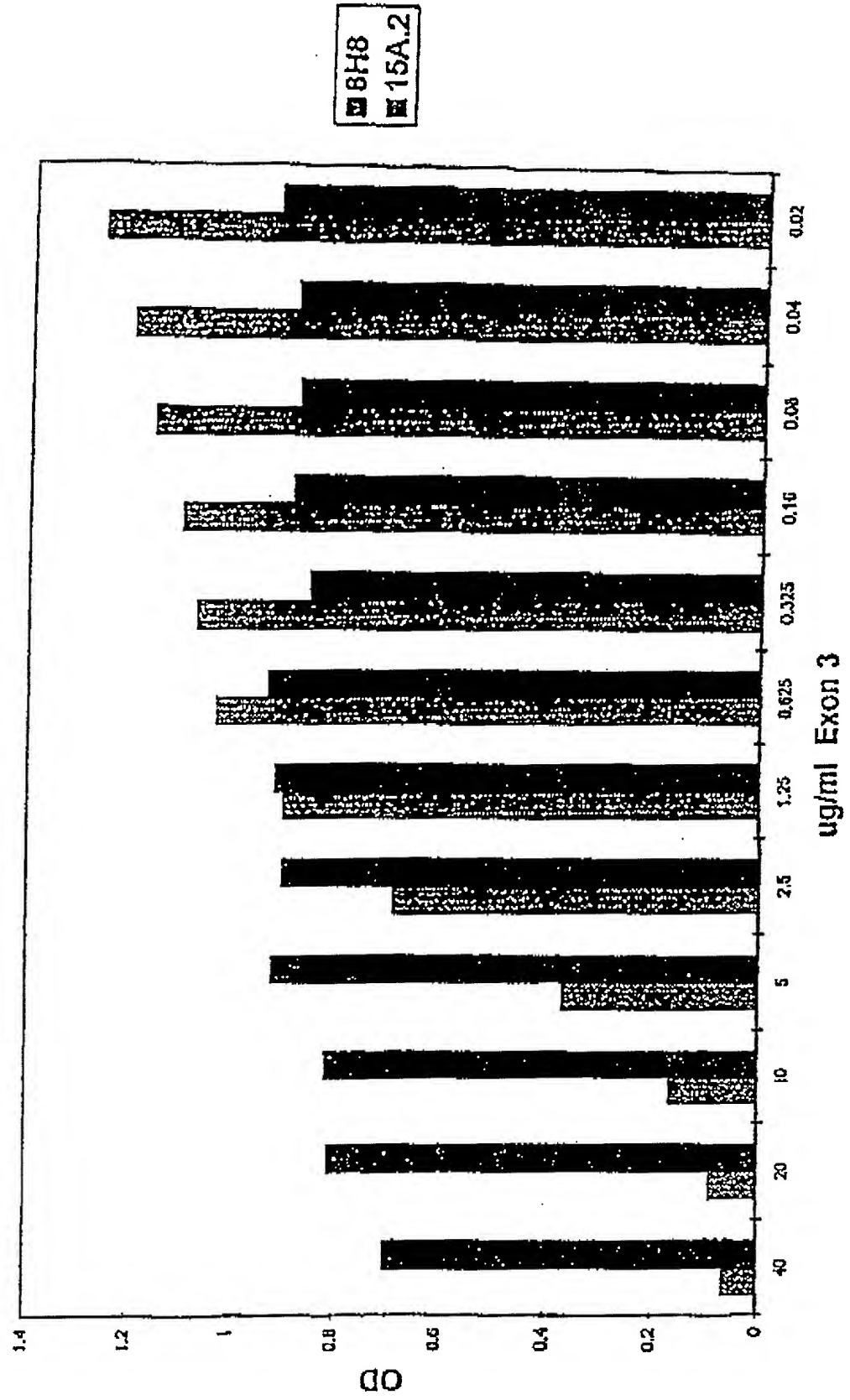


FIGURE 4

Ability of soluble, purified Canine IgE to inhibit Mab 8H8 (2.5 ug/ml) or 15A.2 (2.5 ug/ml) from binding to an IgE solid phase

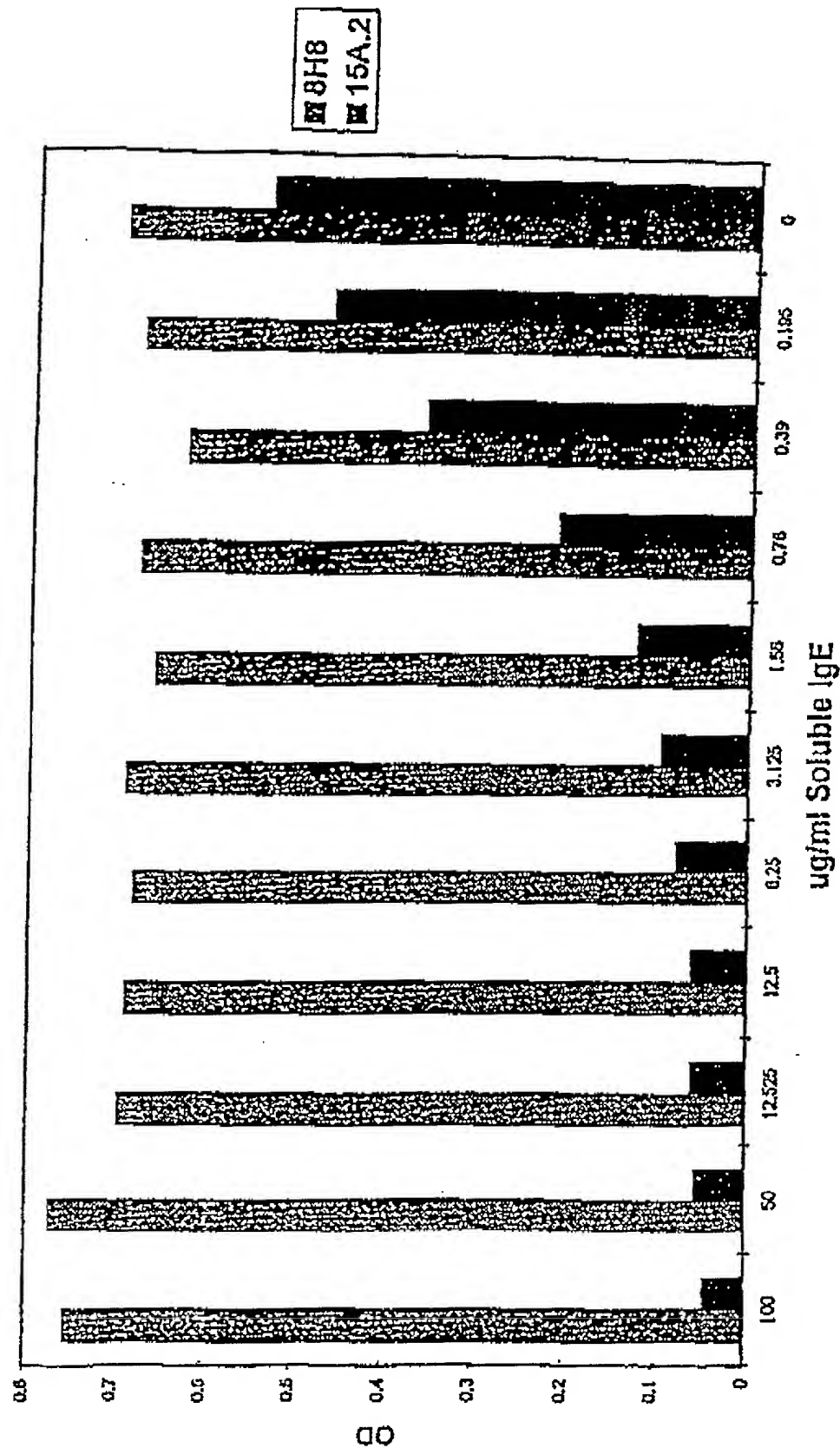


FIGURE 5

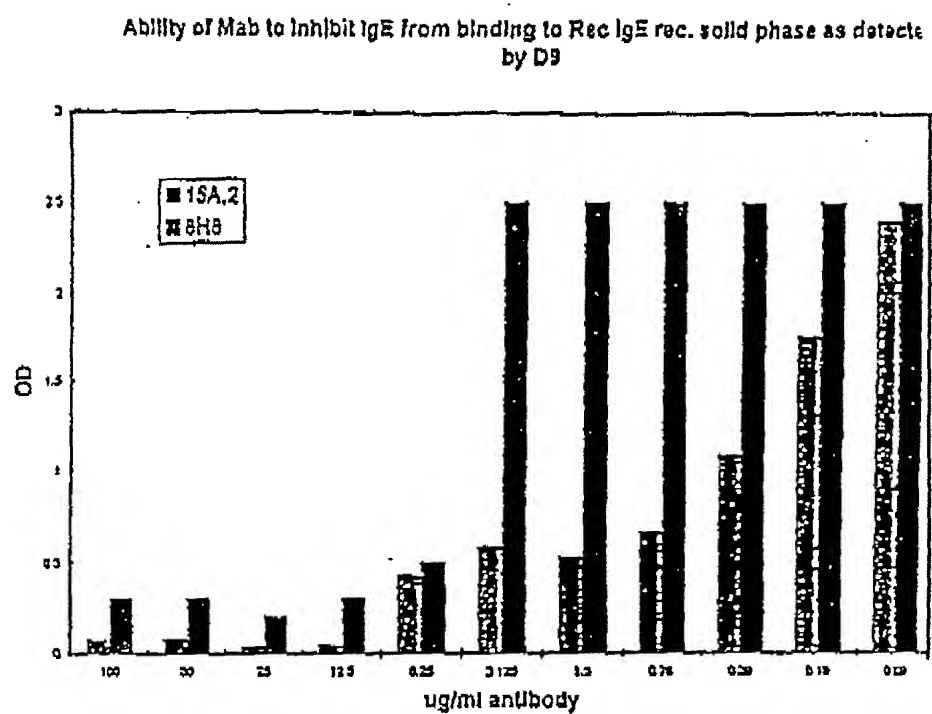


FIGURE 6

LIBRARY	SEQUENCE OF DISPLAYED PEPTIDE	ISOLATE
PhDc7c	C S N P H V T H C	M13 1&9
PhDc7c	C S H P H L T H C	M13 7
PhDc7c	C S N P H I T Q C	M13 10
PhDc7c	C M N P H I T H C	M13 14
PhDc7c	C T N P H N P Y C	M13 2&8
PhDc7c	C P N P H N P Y C	M13 3&5
PhD12	V T L C P N P H I P M C	M13 48
PhDc7c	C H P H L P K S C	M13 4&12
PhDc7c	C H P H L P K R C	M13 6
	Y C R V T H P H L P K D I V R S I	Canine IgE

FIGURE 7

IgE ALIGNMENT 15A.2 EPI TOPE															SPECIES				
Y	C	R	V	T	H	P	H	L	P	K	D	I	V	R	S	I	Canine		
Q	C	R	V	T	H	P	H	L	P	R	A	L	M	R	S	T	Human		
Q	C	R	V	T	H	P	H	L	P	R	A	L	V	R	S	T	Green Monkey		
Q	C	K	V	T	H	P	D	L	P	L	V	I	V	R	S	I	Cat		
Y	C	N	V	T	H	P	D	L	P	K	P	I	L	R	S	I	Swine		
Q	C	I	V	D	H	P	D	F	P	*	*	I	V	R	S	I	Mouse		
K	C	T	V	S	H	P	D	L	P	R	E	*	W	R	S	I	Horse		

* Denotes a deletion

FIGURE 8

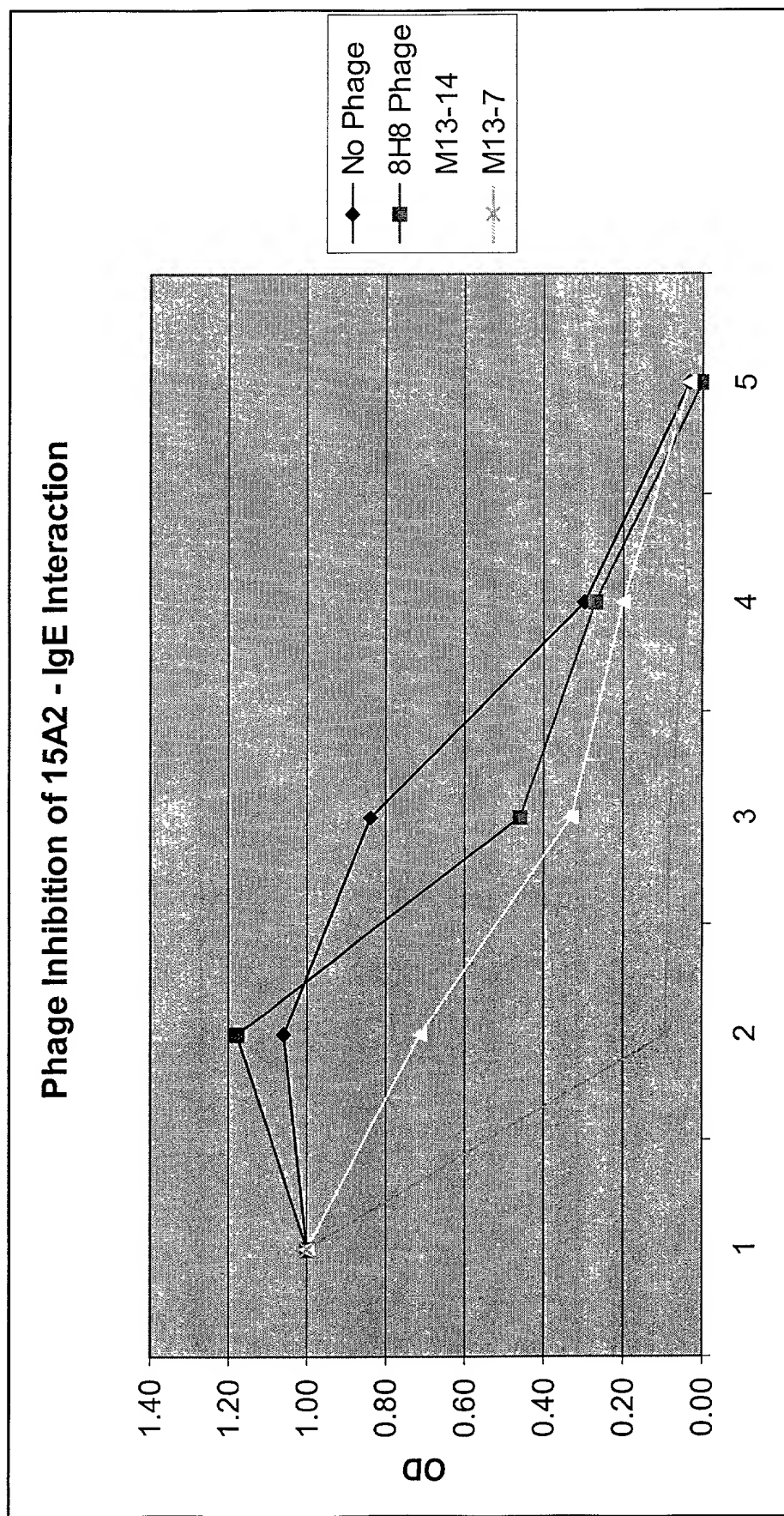


FIGURE 9

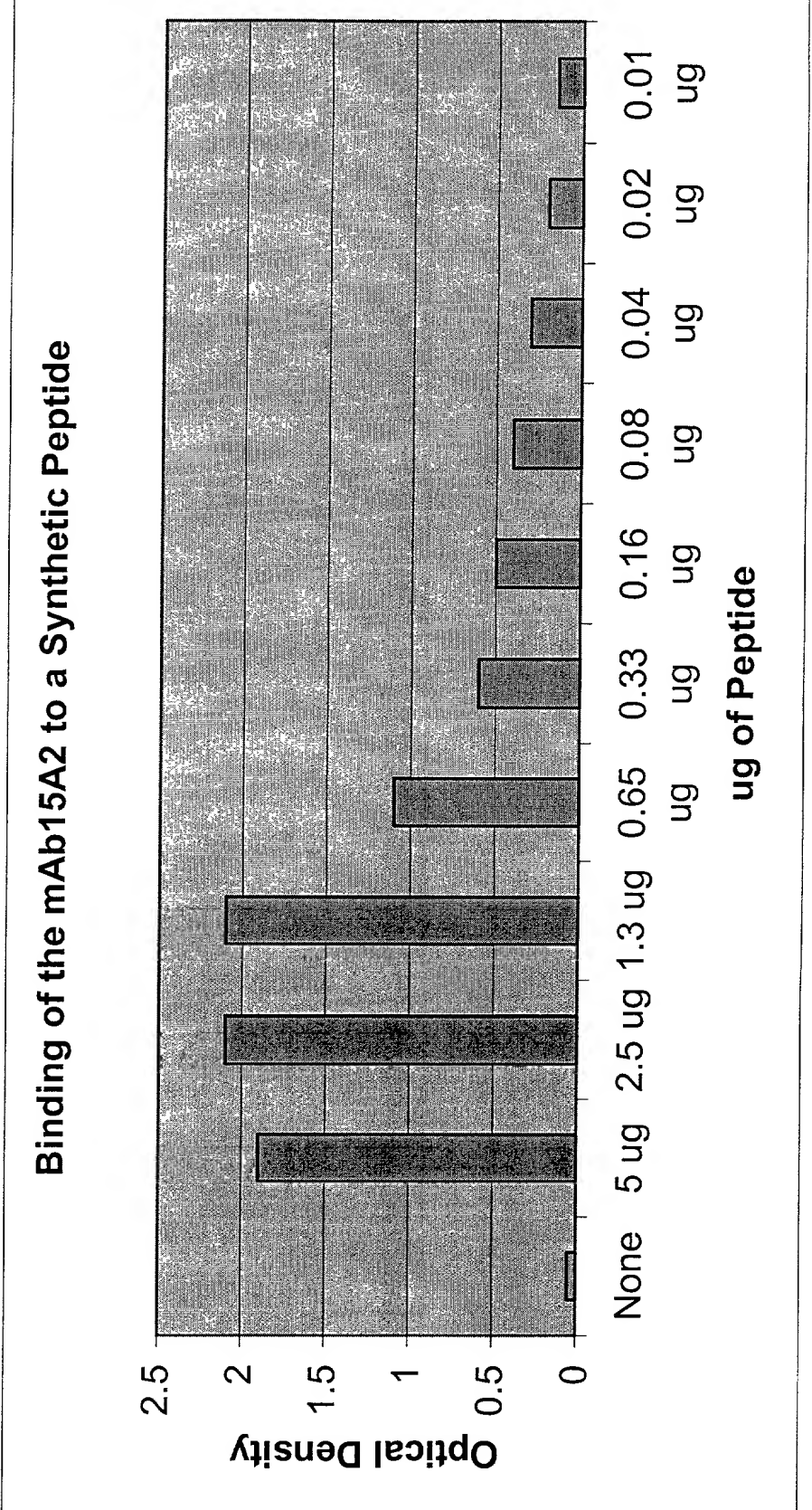


FIGURE 10A

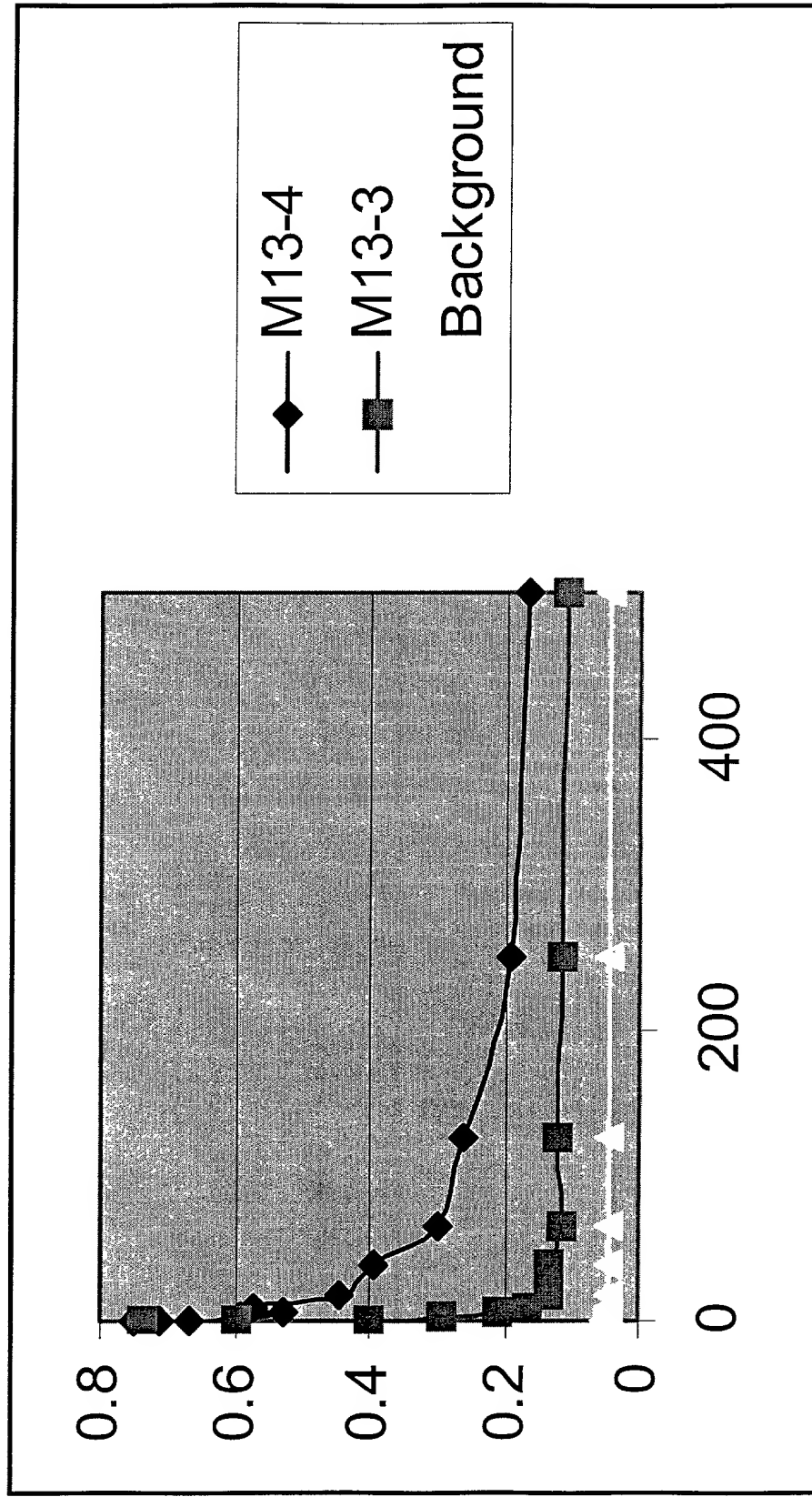
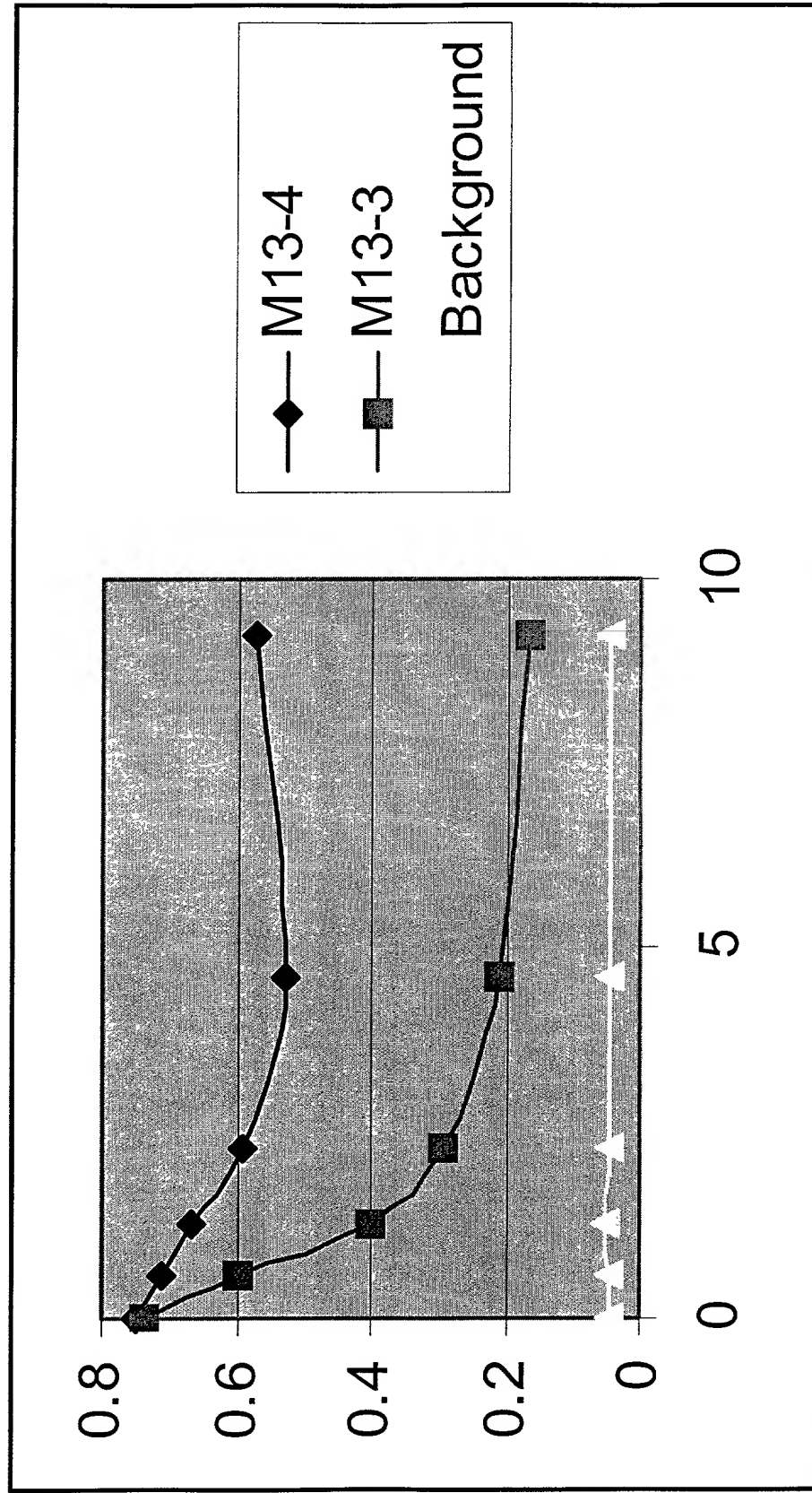


FIGURE 10B



000007 09270000

FIGURE 11

Malb conjugate titration on purified canine IgE solid phase

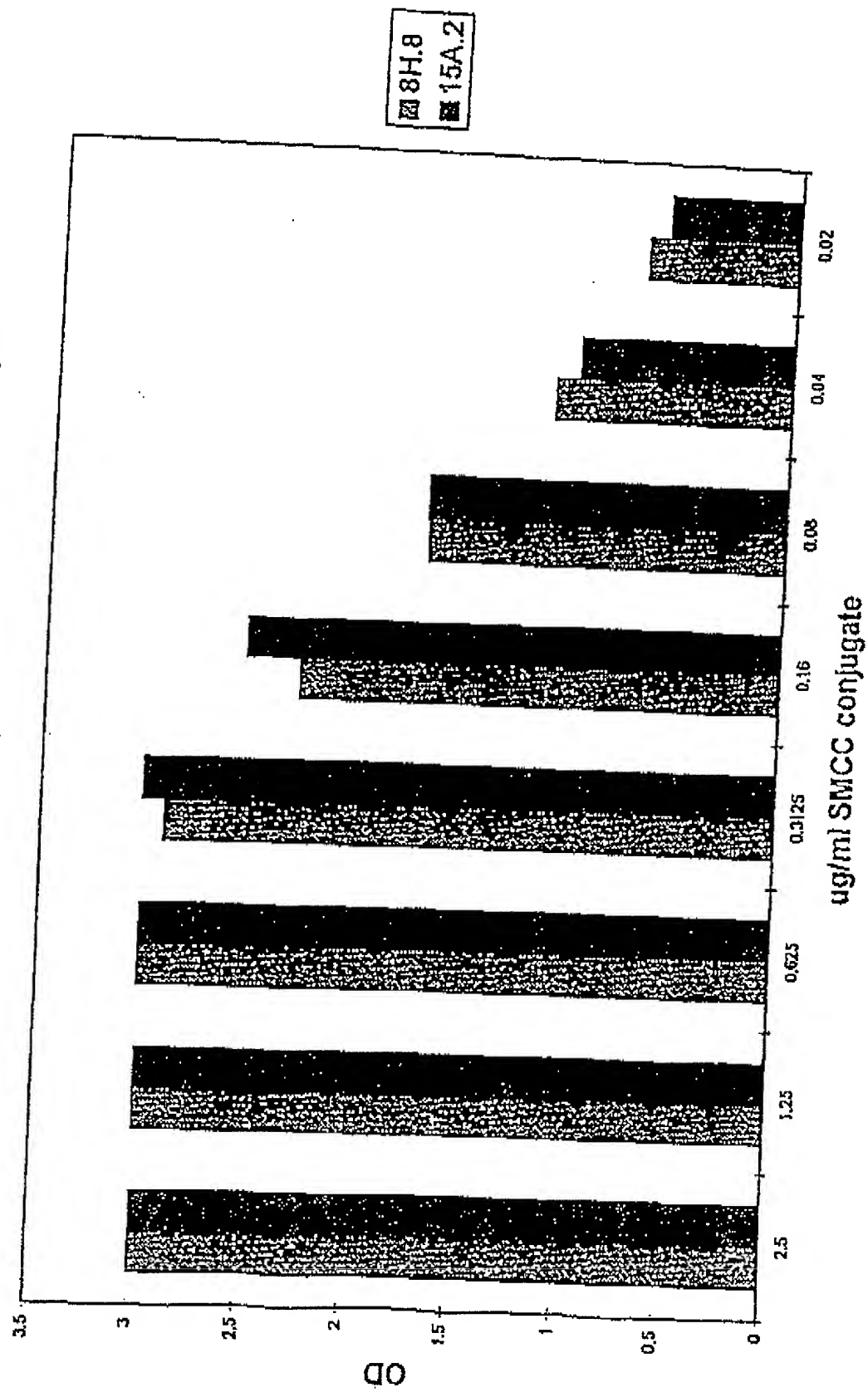


FIGURE 12

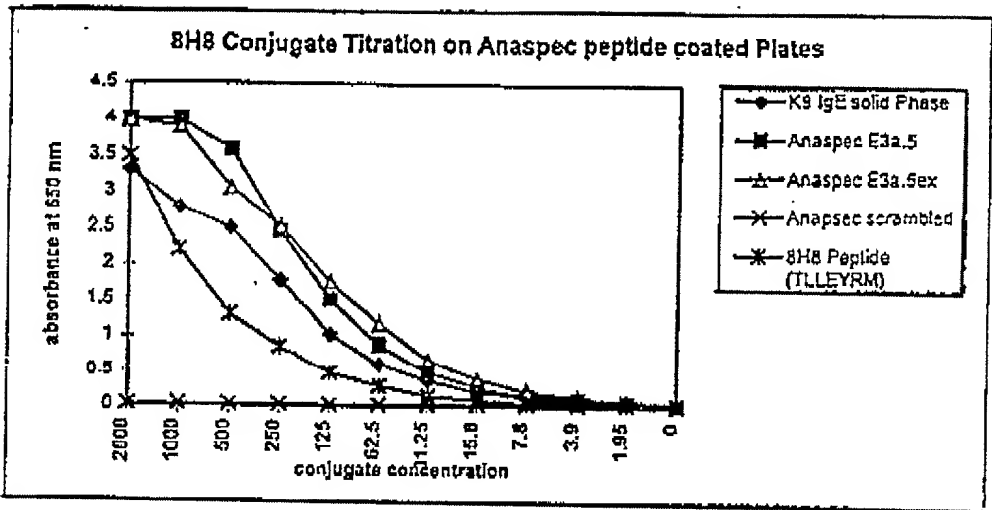


FIGURE 13

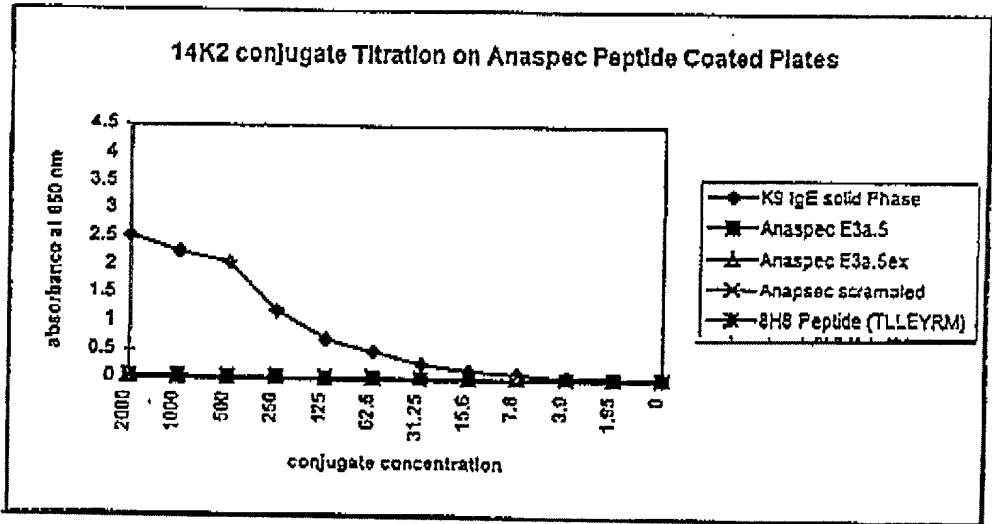


FIGURE 14

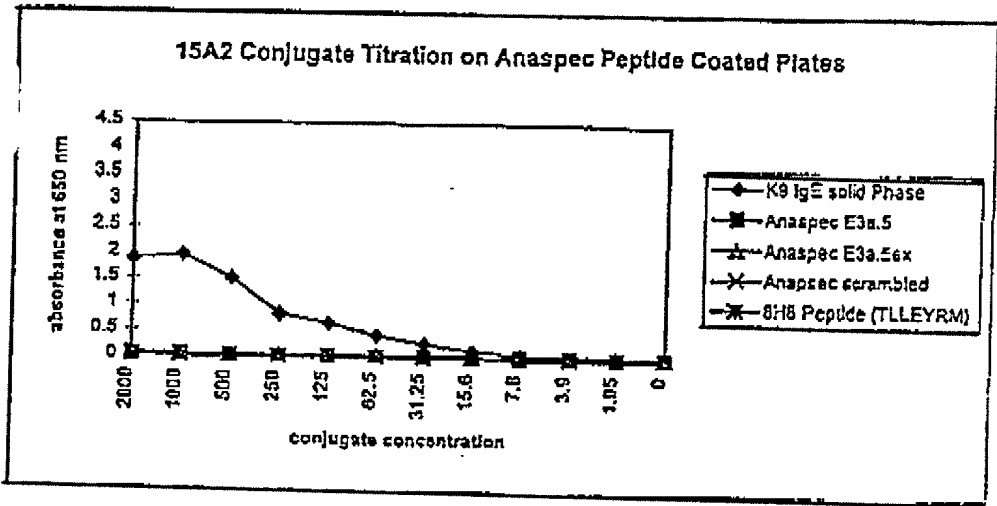
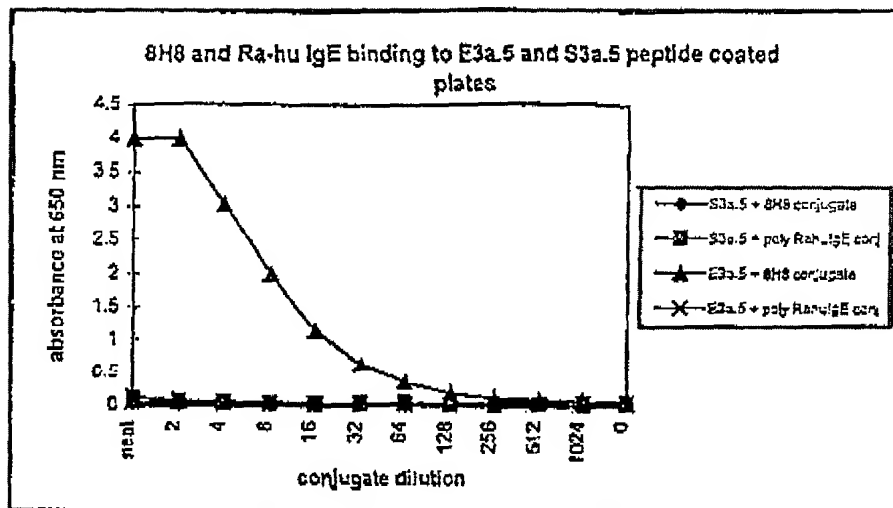


FIGURE 15



Downloaded from www.sagepub.com at 03/24/2015

FIGURE 16

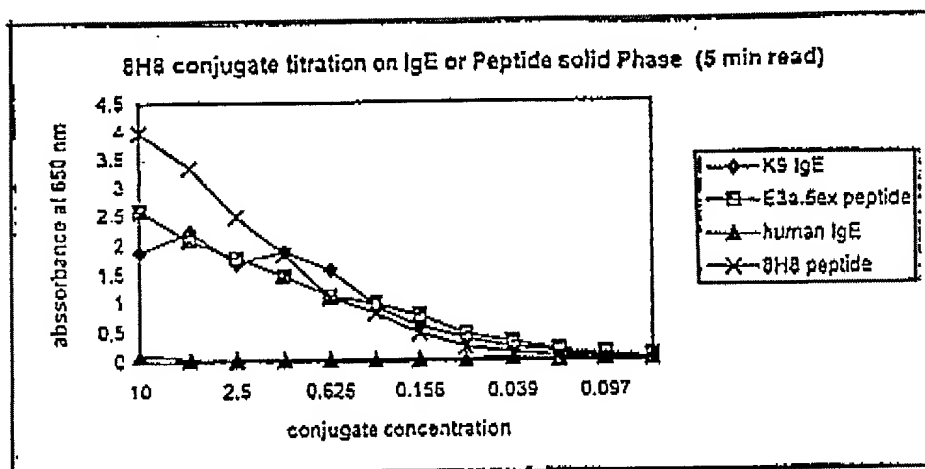


FIGURE 17

